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COMPARATIVE VASOMOTOR REACTIONS IN BRANCHES OF THE ARTERIAL TREE

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That arterial pressure varies in different portions of the arterial tree has long been known. Hürthle (1) after a series of experiments on dogs, cats and rabbits came to the conclusion that the mean end pressure in the carotid artery was always slightly higher than that in the femoral artery. Earlier Volkman (2) reported that the systolic end pressure of the femoral artery was greater than that of the carotid artery. Poiseuille (3) early and Dawson (4) later, have shown that the mean pressure is relatively constant throughout the larger branches of the arterial tree. That the systolic end pressure, as measured by the usual methods decreases with the increase in distance from the heart (and with possibly the decrease in the diameter of the artery) is a well known fact and easily demonstrable.

The technique used in this laboratory of taking a series of blood pressure records as an index to the condition of the vasomotor apparatus of an animal, has of necessity demanded the use of a different branch of the arterial tree in each successive determination. Those used have been right and left femoral, right and left subclavian and right and left carotids. The systolic end pressure under the influence of varying doses of adrenin, nicotine and sometimes pituitrin were used to test the vasomotor irritability. Since this systolic end pressure is known to vary in the different arteries, this question arose: does the arterial pressure throughout the arterial tree rise the same number of millimeters of mercury or does the rise bear a constant ratio to the normal systolic pressures occurring in the individual arteries? That the rise is constant had been assumed as demonstrated but a recent search of the literature has failed to reveal any definite grounds for the supposition. A priori it might be thought that the factors which cause variations in the systolic pressure might cause also a difference in the pressor or depressor responses to drugs. The matter should be determined by direct experimentation. It has been partly answered in this laboratory in a series of experiments by Hoskins and Wheelon (5) from an extended study of the variability of blood pressure and of vasomotor irritability in the anesthetized dog. They, however, took their records from but one artery at a time. They observed that the blood pressure maintained a fairly constant level and that the rises in pressure induced by intravenous injections of pressor drugs were also fairly constant for equal injections. Whether a difference in reaction in the different arteries contributed any variability to their results was not determined

METHOD

To decide the point, normal dogs were anesthetized in the usual manner with ether. Hall reservoir cannulas of essentially the same size were inserted into the different arteries. These were connected by equal lengths of uniform rubber tubing with the large Harvard mercury manometers also of practically the same bore. The mercury columns stood at 12.5 cm. in each manometer and the bases of the manometers were on the same level.

Injections of adrenin, nicotine and pituitrin were used to stimulate the vasomotor system. For the injection of these a venous cannula was connected by a short piece of rubber tubing with a 100 cc. burette containing 0.8 per cent sodium chloride solution. The drug solutions were injected by hypodermic syringe into the rubber tubing above a clamp "shut off" and then run into the vein with 10 cc. of the salt solution.

The blood pressures were recorded on separate drums on which they were measured without any reference to each other. The data were later segregated and compared.

RESULTS

The mean pressures in the different arteries used were found to vary more than the literature on the subject would lead one to expect. They differed usually by from 8 mm. to 40 mm. of mercury. In one case there was a very unusual difference of 120 mm. of mercury in different arteries of the same dog. This was proved beyond question but the cause was not determined.

In no case was there a significant difference in the amount of rise regardless of whether the rise be sharp or gradual, of a few mm. of mercury or many. The results obtained from one experiment are given below. The pressure noted as "systolic" is the highest indicated by the mercury manometers. The actual damping effect of the instruments was not determined.

Simultaneous rise of blood pressure in different arteries

	RIGHT FEMORAL ARTERY	RIGHT CAROTID ARTERY	SUPERIOR MESENTERIC ARTERY
	Systolic B. P., 96 mm. Mean, 95 mm.	Systolic B. P., 116 mm. Mean, 110 mm.	Systolic B. P., 103 mm. Mean, 102 mm.
	mm.	*mm.*	*mm.*
1 cc. Adrenin	34	34	34
1 cc. Adrenin	40	42	42
2 cc. Adrenin	34	38	38
2 cc. Adrenin	27	26	28
1 cc. Nicotine	48	48	48
1 cc. Nicotine	35	36	35
2 cc. Nicotine	28	30	28
2 cc. Nicotine	29	30	30

Even more striking was the constancy of reaction in the animal which showed the greatest difference in systolic pressure. For instance 2 cc. of adrenin caused the pressure in the carotid artery to rise from 220 mm. to 258 mm. while in the femoral artery it went from 98 mm. to 136 mm. Exactly 38 mm. in each case.

Attempts were then made to determine how far toward the periphery of the arterial branches this constancy persisted. Capillary cannulas were made with which arteries as small as 0.5 mm. in diameter could be utilized.

It was found that if cannulas of the same size were used, one in a branch 0.5 mm. in diameter and another in a branch 5 mm. in diameter, the rises and falls in pressure maintained the same constancy. If, however, the cannulas were not of the same size the rises bore an approximately direct ratio to the sizes of the cannulas.

Later the depressor reactions induced by small doses of adrenin and of the nitrites were compared and found to be as constant as the pressor reactions.

These results show that a sudden rise or fall in arterial pressure is immediately transmitted to all proximal parts of the greater arterial system to the same extent regardless of the existing pressure in the artery from which it is measured.

The outcome of the experiments presents an interesting problem in mechanics. The fact that different systolic and mean pressures exist in various arteries under a constant head of pressure indicates that the peripheral resistance to outflow is variable in the different branches. The augmentation of the resistance produced by the pressor drugs might be expected also to vary in different branches, thus producing a similar difference in the pressor effects, but such is not the case. This fact might be supposed to be correlated with the briefness of the reaction, whereas a longer acting pressor influence might allow an equilibrium to be established and a differing rise sustained. The use of pituitrin, however, shows that such is not the case; that irrespective of the duration of the reaction the pressor effect is the same even when there is a marked difference in the initial pressures.

SUMMARY

Vasomotor reactions, pressor and depressor, in branches of the arterial tree which may be of great variations in diameter and of varying initial systolic and mean pressures, are equal. This can easily be demonstrated provided there is no essential difference in the size of the cannulas used to measure such responses.

I wish to express my sincere appreciation of the suggestions and criticisms of Dr. R. G. Hoskins during the preparation of this paper.

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THE BONE MARROW AS A SOURCE OF PROTHROMBIN

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I. INTRODUCTION

The modern history of blood coagulation begins with the work of Alexander Schmidt in 1861. Since that time a vast literature has grown about the subject, indeed has reached such a size that it is a confusing task to cover the work upon one element in, or one phase of the process. The present investigation concerns itself with the origin of prothrombin from the bone marrow. This substance forms one of the indispensable factors for coagulation. Its necessity is recognized by all theories for the explanation of this process and the technique which we have employed for the demonstration of its presence is equally acceptable to all theories. Morawitz (1, 2), in 1904 and 1905 has reviewed the literature upon the origin of prothrombin or thrombogen or fibrin ferment as the substance is variously called. We shall mention no specific work published prior to these articles, confining ourselves to a statement of the views which existed in 1904 and their modification up to the present date. Such views may be grouped briefly as follows:

(a) Prothrombin arises from all the formed elements of the blood.

(b) Prothrombin arises from the leucocytes.

(c) Prothrombin arises from the platelets:

1. Which are produced by leucocytes and erythrocytes;
2. Which are independent elements.

(d) The formed elements of the blood have nothing to do with clotting.

(e) Prothrombin cannot be obtained from blood free tissue so its source of origin must be in the blood itself.

Formed elements of the blood. Howell (3) in his work on the coagulation of lymph collected from the thoracic duct can not decide finally upon the presence of prothrombin in the lymphocytes. He concludes that if lymphocytes do contain this substance it is in extremely small amounts. Further evidence against the origin of prothrombin from this source is found in the failure of attempts to obtain it from extracts of lymph glands. Such efforts have been very carefully made by Nolf (4, 5).

Work upon other colorless corpuscles of the blood, mast cells and those indefinite types classed as large mononuclears, has been impossible since these cells can not be collected in sufficient numbers to permit study.

There is no doubt that thoroughly washed mammalian erythrocytes do not yield prothrombin. The nucleated red corpuscles of lower forms are equally deficient. Study of isolated normoblasts of the mammal has been impossible, but in our series of experiments we have found that there is no parallelism between the prothrombin content and the number of normoblasts in the fluids we have tested.

Leucocytes. Morawitz (1) has studied the question of the origin of prothrombin from the leucocytes. After aleuronat injections he examined the washed polymorphonuclear leucocytes so obtained, and found their prothrombin content practically nil. This was the first satisfactory direct examination of these elements, former conclusions being based on the fact that in coagulation there is probably some leucocyte destruction. Nolf (4) also finds no prothrombin in the leucocytes but does get evidence of a large amount of thromboplastic material, which in this particular case he calls leucothrombin, and later thrombozyme (5). Bordet and Delange (6) find a large amount of thromboplastic substance in the leucocytes but nothing that corresponds with any certainty to prothrombin. Their experiments have been confirmed by Lee and Vincent (7).

The question of prothrombin origin from young forms of the leucocytic series has not been adequately investigated. In our own experiments when myelocytes have been numerous in the fluid tested there has been no significant increase in the amount of prothrombin.

Blood platelets. There is a growing conviction that in the adult mammal plates do not arise from leucocytes nor from erythrocytes, but following the work of Wright (8) from the megacaryocytes of the bone marrow. Ogata (9), Bunting (10), Downey (11), and very recently Ferrata and Negreiros-Rinaldi (12), have given Wright's work the most ample histological verification. The absence of prothrombin from erythrocytes and from all forms of white cells encourages this view. Morawitz (1) obtained platelets in pure form by differential centrifugalization and showed them to be rich in prothrombin. Nolf (5) found only a moderate amount of prothrombin in the plates. Bayne-Jones (13) has completely confirmed the work of Morawitz.

Formed elements of blood not concerned in coagulation. These demonstrations—and they might be multiplied—of large amounts of prothrombin in the plates dispose effectively of the idea that the formed elements of the blood have nothing to do with clotting. We know definitely from the work of Deetjen (14) and others that disintegration of the plates accompanies the act of coagulation and indeed is one of the first features in it.

Origin of prothrombin from tissues other than the blood. Nolf (5) and Rettger (15) have found traces of prothrombin in extracts made from the spleen. Rettger obtained what he believed to be a possible trace from washed bone marrow. With the exception of the liver no other tissues have been shown to contain prothrombin. Nolf (16) believes that nearly the whole supply of prothrombin is formed by the liver, basing his belief upon the disappearance of fibrinogen and of prothrombin from the circulation when the liver is removed.

Selling (17) first demonstrated extensive bone marrow destruction in benzol poisoning. Duke (18) found an extremely low platelet count in this condition and ascribed the bleeding tendency to this deficiency. Hurwitz and Drinker (19) carried these observations a step further by finding an agreement between the platelet loss so produced and the quantity of prothrombin in the blood.

We may say in summary, therefore, that prothrombin has been shown to exist in the blood plates, and that these elements originate from the megacaryocytes of the bone marrow. The evidence, however, that the bone marrow itself as a distinct tissue contains prothrombin is very slight. So far as is known other tissues, with the possible exception of the liver and of the spleen, have no significant part in the manufacture of this element.

While devising a method of perfusing bone marrow, the opportunity presented itself of studying the prothrombin content of the perfusing fluid after its passage through the marrow. The discovery of an unexpectedly large amount of prothrombin in this fluid led the authors to make careful studies of specimens of fluid taken at frequent intervals during the course of perfusions lasting 3 to 6 hours, with respect to their content of cells and of prothrombin. The results of these studies, which are reported in detail in this paper, lead to the conclusion that the bone marrow is an important and specialized source of prothrombin.

B. Method of testing specimens for prothrombin

The method of determining the amount of prothrombin in the various specimens differed according to the perfusing fluid. When Ringer's solution was used the prothrombin tests were made by adding to a given amount of a fibrinogen solution in a series of test tubes, a definite amount of each specimen, and determining the time required for the first signs of clotting; e.g., *fibrinogen*, 0.5 cc.; *specimen 1*, 1.0 cc.; *coagulation time*, 4 minutes. The calcium in the Ringer's solution was sufficient to convert all of the prothrombin in the different specimens into thrombin, which substance then reacted with the fibrinogen to form fibrin. In every experiment each prothrombin test was made in duplicate.

When 0.9 per cent sodium chloride was used as the perfusing fluid it became necessary to add calcium as well as fibrinogen to the specimens before clotting could occur. This was accomplished by adding a certain number of drops of a 0.5 per cent calcium chloride solution to each test tube containing the required amount of fibrinogen, before any of the specimen to be tested was added; e.g., *fibrinogen*, 0.5 cc.; *0.5 per cent* $CaCl_2$, 2 drops; *specimen 1*, 1.0 cc.; *coagulation time*, 9 minutes.

When sodium oxalate made up in physiological saline solution was used as the perfusing fluid, the same procedure of adding calcium chloride to the series of tubes was followed but in these cases it was necessary to add an amount of calcium, sufficient not only to precipitate out all of the oxalate in the solution, but sufficient also to convert all of the prothrombin present into thrombin. The amount of calcium necessary to accomplish these two purposes was determined in each experiment.

The fibrinogen solutions used in these tests were made from the plasma of cats by Howell's modification of the Hammarsten method of precipitation by half saturation with sodium chloride (21). A fresh solution of fibrinogen was made in nearly every case on the day before the experiment, since solutions of isolated fibrinogen deteriorate in a few days. Each solution of fibrinogen was tested immediately before being used with respect to its clotting power when added to a solution of thrombin (22). No fibrinogen solution was used unless 0.5 cc. of it when added to 0.2 cc. of thrombin solution gave a clot in 5 minutes or less.

C. Method of testing for fibrinogen

In one experiment a certain amount of a thrombin solution was added to a definite amount of each specimen of perfusing fluid to determine by the appearance or non-appearance of clots whether or not the specimens contained any fibrinogen; e.g., *specimen,* 0.7 cc.; *thrombin,* 0.2 cc.; *coagulation time,* no clot in four hours. No clots appeared in any case. This led the authors to conclude that the bone marrow is probably not a source of fibrinogen.

D. Other studies made on marrow perfusates

In each perfusion specimen a count was made of the number of red blood cells present and of the number of white cells. Smears and differential counts were also made. Sections were made of the normal and of the perfused bone marrow with the view of studying the effect of perfusion on the histological appearance of this structure. The difficulty, however, of making satisfactory sections of the marrow of the dog because of the cancellous tissue which enmeshes the red marrow, made these histological studies unsatisfactory.

E. Types of control experiments

Three types of control experiments were performed.

1. Experiments to determine the amount of prothrombin demonstrable in normal bloods of various cellular concentrations, with and without the addition of thromboplastic substance.
2. Experiments to determine whether or not solutions dripping over the surface of a fresh naked bone acquire any prothrombin.
3. Experiments to determine whether or not prothrombin is contained in fluids perfused through organs other than the bone marrow.

III. EXPERIMENTAL RESULTS

A. Perfusions with Ringer's solution and with 0.9 per cent sodium chloride

In the early experiments perfusions were made with Ringer's solution, the tibias perfused being stripped of muscles, tendons, etc., and then placed in a glass chamber as previously described. Specimens obtained in this manner all showed the presence of considerable amounts of prothrombin though some bones yielded more than others. The following table compiled from the results of Experiment XXIII illustrates the usual findings in this type of experiment.

TABLE 1

Experiment XXIII (Ringer's solution)

	SPECIMENS							
	1	2	3	4	5	6	7	8
Prothrombin time in minutes	4	12	28	38	32	46	36	41
Red blood cells per c. mm.	3500	1500	500	600	700	800	850	1650
White blood cells per c. mm.	150	200	90	50	60	100	110	135
Rate of flow in cc. per minute	6.4	6.4	5.9	4.5	3.6	3.0	2.3	1.6

In Ringer perfusates all the prothrombin present is activated to thrombin by the calcium in the solution. In the early specimens which contain a certain amount of blood, this prothrombin may encounter enough fibrinogen to cause the formation of small clots. In this way thrombin is used up and less of it is demonstrable in the collected specimens. For this reason, after using Ringer's solution in a number of cases, it was found more satisfactory to perfuse with 0.9 per cent sodium chloride. Under these circumstances there is no possibility of clotting until the investigator adds both calcium and fibrinogen at the time of making his tests. The sodium chloride perfusates in all cases were rich in prothrombin, as the following table taken from Experiment XI will testify.

TABLE 2

Experiment XI (0.9 per cent NaCl)

	SPECIMENS										
	1	2	3	4	5	6	7	8	9	10	11
Prothrombin time in minutes	9	18	32	47	41	50	53	54	78	44	49
Red blood cells per c. mm.	3400	1700	1500	1320	2300	1400	1200	1000	1600	1300	1800
White blood cells per c. mm.	0	150	200	350	550	260	500	600	500	1200	600
Rate of flow in cc. per minute	8.2	9.8	8.2	3.2	7.2	8.1	8.3	7.6	4.1	6.1	6.0

B. Control experiments: possible sources of prothrombin other than the marrow

(1) *Blood.* In an examination of these tables the question naturally arises—Is the small amount of blood which these specimens contain the source of the prothrombin in them? In Experiment XI (table 2) blood was allowed to flow from the carotid artery of the dog whose tibia was being perfused into a large volume of 0.9 per cent sodium chloride until the color of the salt solution closely resembled that of the first specimen of perfusing fluid.

A count was then made of the number of red cells in this diluted blood and a determination of the time required for clotting after the addition of a given amount of calcium chloride and of fibrinogen—this latter maneuver constituting our usual test for prothrombin; e.g., *fibrinogen*, 0.5 cc.; *0.5 per cent* $CaCl_2$, 2 drops; *diluted blood*, 1.0 cc.; *coagulation time*, no clot in 3 hours, 49 minutes.

The number of red cells per c. mm. in the diluted blood was 3500: the prothrombin test gave no clot in 3 hours and 49 minutes. The first specimen of marrow perfusate taken from the same dog showed 3400 red cells per c. mm., approximately the same number as in the diluted blood; while the prothrombin test in this specimen gave a clot in 9 minutes.

We next prepared from Dog XI a second lot of diluted blood in which the red cell concentration was determined to be 25,320 cells per c. mm. The prothrombin time in this case was 34 minutes. These figures in their turn should be contrasted with the red cell count of 3400 per c. mm. and the prothrombin time of 9 minutes in the first specimen of the perfusing fluid.

These observations on diluted blood furnish definite evidence that the small amounts of blood present in the specimens of fluid which have passed through the marrow are not the source of the prothrombin in them.

In order to furnish still further evidence on this point Experiment XIII was performed in which a given amount of oxalated blood was diluted with 0.9 per cent sodium chloride through a series of progressively increasing dilutions. At each dilution a red cell count and a white cell count was made, and the prothrombin time was determined in the usual manner. In this way we determined approximately the normal prothrombin times for bloods of different cellular concentrations. When these normal prothrombin times are compared with the prothrombin times of specimens of Ringer's solution or of 0.9 per cent sodium chloride which have passed through the marrow, it will be found in every case that the marrow perfusates contain much greater amounts of prothrombin.

TABLE 3

Experiment XIII (Blood diluted with 0.9 per cent NaCl)

	SPECIMENS									
	1	2	3	4	5	6	7	8	9	10
Dilution: Blood to 0.9 per cent NaCl..........	1: 10	1: 20	1: 40	1: 80	1: 160	1: 320	1: 640	1: 1280	1: 2560	1: 5120
Prothrombin time in minutes............	3	5	8	16	37	69	153	no clot in 6 hrs.	no clot in 6 hrs.	no clot in 6 hrs.
Red blood cells per c. mm.....	462,000	261,000	94,640	66,000	31,800	14,648	10,348	5748	4448	3450
White blood cells per c. mm...........	1496	848	340	200	70	15	4	0	0	0

Table 3 summarizes the figures obtained in Experiment XIII. But it must be remembered that in many of the experiments the specimens of perfusing fluid dripped over the surface of the bone before they were collected. In this way each specimen received an increment of thromboplastic substance.

In order to simulate this state of affairs in diluted blood a second lot of oxalated blood was diluted with water in a series of dilutions corresponding to the 0.9 per cent sodium chloride dilutions just described. The water at once caused complete hemolysis with the liberation of large amounts of thromboplastic substance. Since it was not possible in this series to count the number of red cells at each dilution, the concentration of blood in each specimen was estimated from the corresponding sodium chloride specimen.

The prothrombin times in the water diluted bloods were then tested in order to determine the effect of the increment of thromboplastic material on the rate of clotting. The figures obtained in this series may be seen in table 4.

But even these shorter prothrombin times contrast markedly with the still shorter prothrombin times in marrow perfusates of the same red cell concentration. Take for example in table 4 the specimen of

TABLE 4

Experiment XIII (Blood diluted with H_2O)

	SPECIMENS									
	1	2	3	4	5	6	7	8	9	10
Dilution: Blood to H_2O.........	1: 10	1: 20	1: 40	1: 80	1: 160	1: 320	1: 640	1: 1280	1: 2560	1: 5120
Prothrombin time in minutes............	1.5	2	3	4	5	12	21	41	77	222
Estimated red blood cells per c. mm..........	462,000	261,000	94,640	66,000	31,800	14,648	10,348	5748	4448	3450
Estimated white blood cells per c. mm..........	1496	848	340	200	70	15	4	0	0	0

water diluted blood which contained 3450 red cells per c.mm. The prothrombin test on this blood gave a clot in 222 minutes. Perfusion specimen 1 of Experiment XI contained 3400 cells per c.mm.; the prothrombin time was 9 minutes. These observations seem to us to prove conclusively that while the dilute blood present in the perfusion specimens undoubtedly adds a trace of prothrombin to these liquids, it is certainly not the chief source of the prothrombin which they contain.

(2) *Surface of the bone.* After eliminating the blood as the chief source of the prothrombin in the marrow perfusates, we next investigated the question as to whether or not salt solution which has not passed through the marrow but which has dripped over the surface of a fresh bone, contains any prothrombin. Experiments on this point demonstrated that a solution dripping slowly over a freshly stripped bone picks up small amounts of blood and in this way acquires traces of prothrombin. That the amounts of prothrombin acquired in this way are very small may be seen from table 5 (Exp. XV) which should be contrasted with table 2 (Exp. XI), showing the prothrombin present in marrow perfusates.

In order to eliminate entirely the factors involved when the perfusing fluid drips over the bone, a series of experiments was performed in which the bone was not removed from the animal after the establishment of perfusion. The technique of this preparation has already been described. The disadvantage to this method of collection is the fact that the specimens are apt to contain more blood than specimens obtained in the manner first described. This disadvantage prevented us from using exclusively this method of collection. The

TABLE 5

Experiment XV (0.9 per cent NaCl allowed to drip over bone)

	SPECIMENS					
	1	2	3	4	5	6
Prothrombin time in minutes	40	1 hr. 42 min.	no clot after 2 hrs. 47 min.	no clot after 2 hrs. 27 min.	no clot after 2 hrs. 17 min.	no clot after 2 hrs. 3 min.
Red blood cells per c. mm.	4400	848	448	50		
White blood cells per c. mm.	50	14				
Rate of flow in cc. per minute	4.3	7.0	8.6	3.6		

results obtained from prothrombin tests on perfusing fluids collected directly from the popliteal vein showed that these liquids contained a quantity of prothrombin greater than could possibly be accounted for by the amount of blood in them. The following table taken from Experiment XVI gives a series of figures illustrating this fact.

TABLE 6

Experiment XVI (0.9 per cent NaCl—Specimens collected from vein)

	SPECIMENS														
	1	2	3	4	5	6	7	8	9	10	11*	12	13	14	15
Prothrombin time in minutes	14	30	13	7	5	9	11	4	16	20	11	37	28	21	28
Red blood cells per c. mm.	61,800	38,800	16,800	22,960	19,440	10,960	9280	7000	5648	2248	20,160	5240	4240	4000	3600
White blood cells per c. mm.	150	250	700	500	600	400	300	250	200	250	700	550	200	548	250
Rate of flow in cc. per minute	7.2	6.0	3.0	1.4	1.2	1.3	1.3	1.3	1.2	1.3	1.7	1.0	1.2	1.3	1.5

* This specimen contained a clot which had caught in the rubber tubing and had collected cells around it.

(3) *Other organs. Prothrombin content of 0.9 per cent sodium chloride perfused through liver and spleen.* In order to determine whether or not liquids perfused through organs other than the bone marrow show a content of prothrombin, perfusions were made of the spleen of a dog and of the liver of a guinea pig and the perfusing fluids examined for prothrombin. The results in the case of the spleen were negative.

The traces of prothrombin found could be accounted for by the blood present. In the liver on the contrary a certain small amount of prothrombin was found which could not be due to blood.

C. Possible sources of prothrombin obtained from bone marrow

The question which next arose in this series of experiments was—What cells in the bone marrow are the source of the prothrombin? In attempting to answer this question we considered as a possible source each of the following groups of cells:

(*1*) *Leucocytes.*
(*2*) *Lymphocytes.*
(*3*) *Myelocytes.*
(*4*) *Nucleated red cells.*
(*5*) *Megacaryocytes.*
(*6*) *Blood platelets.*
(*7*) *Wandering endothelial cells.*

(1, 2, 3, 4) *Leucocytes, lymphocytes, myelocytes, nucleated reds.* Of these seven groups of cells we easily eliminated the first four as possible sources of prothrombin. In nearly all of our experiments portions of the perfusion specimens were centrifugalized and smears made from the sediments obtained. Differential counts on these smears gave the percentage of the different types of cells present in each specimen. By this means we were able to compare the amounts of prothrombin present with the number of cells of each variety found. We were never able, however, to establish the least parallelism between the amounts of prothrombin present and the number either of leucocytes, lymphocytes, myelocytes, or nucleated red cells. A specimen rich in leucocytes, for example, was often rich in prothrombin, and equally often its prothrombin content was exceedingly low. That these results agree with the work of other investigators may be seen by referring to our introductory discussion of the theories of origin of prothrombin.

(5, 6) *Megacaryocytes and blood platelets.* The possibility of the origin of the bone marrow prothrombin from the megacaryocytes or the platelets deserves careful consideration. According to the work of Wright (8) and others, the platelets arise as buds from the megacaryocytes, and it has been shown that solutions of platelets contain large amounts of prothrombin. We are, therefore, at once confronted with the question—Does the prothrombin which we have found come from the mother megacaryocytes, from the daughter platelets, or from both?

Our first inclination was to suspect the platelets, which we believed had probably disintegrated during perfusion and had yielded their prothrombin to the perfusion liquids. In endeavoring to find proof for this idea we perfused bones with sodium oxalate solutions (made up in 0.9 per cent NaCl), varying in oxalate strength from 0.1 per cent to 0.3 per cent, in the hope of fixing the platelets and so preventing their disintegration. If such a manoeuver were successful, one ought to find no prothrombin in the oxalate perfusion liquids, or at least only traces, and at the same time plenty of fixed plates. As a matter of fact in all the oxalate perfusates (except the 0.1 per cent perfusate which seemed to be too weak to fix the cells), we did find practically no prothrombin. But at the same time the most careful search revealed no fixed plates, although the usual number of red and of white cells appeared in the perfusion specimens. Centrifugalization of these specimens and treatment of their sediment with water (a manoeuver which would cause fixed platelets to disintegrate and yield prothrombin) gave negative results to our prothrombin tests.

These findings seem to point to one of two conclusions: either (a) the platelets are not the source of the marrow prothrombin, or (b) if the platelets are the source of the marrow prothrombin they are fixed in situ by oxalate solutions and are not washed out with the other marrow cells during perfusion.

Further experience with oxalate solutions showed that if these perfusions are continued long enough prothrombin will eventually appear in the specimens. This suggests that the cells of origin of prothrombin are at first fixed in situ by the oxalate but that under the influence of prolonged washing they will eventually go to pieces. Experiments were performed in which bones were perfused with 0.9 per cent sodium chloride for an hour or more and then the perfusing fluid was suddenly changed to an oxalate solution. In an experiment of this sort the saline perfusate contained the usual prothrombin. This prothrombin promptly disappeared when perfusion with an oxalate solution was begun. The reverse type of experiment was performed in which the bone was first perfused with an oxalate solution and then with physiological saline. The results were the same. The oxalate specimens contained no prothrombin while the saline specimens contained gradually increasing amounts as the salt solution slowly washed the oxalate out of the cells in the marrow and brought about their disintegration. The figures obtained in an experiment of this type may be seen in table 7 taken from Experiment XXVI.

TABLE 7

Experiment XXVI (0.2 per cent $C_2O_4Na_2$ changed to 0.9 per cent NaCl)

	SPECIMENS									
	1	2	3	4	5*	6	7	8	9	10†
Prothrombin time in minutes...........	no clot in 4 hrs.	no clot in 4 hrs.	no clot in 4 hrs.	no clot in 4 hrs.	1 hr. 40 min.	1 hr. 21 min.	1 hr. 9 min.	55 min.	33 min.	18 min.
Red blood cells per c. mm......	6900	5600	4800	1300	1350	900	1200	1000	1100	3300
White blood cells per c. mm............	4000	2500	1300	500	100	250	50	50	43	3100
Rate of flow in cc. per minute.	8.4	6.0	3.8	2.2	2.1	2.1	2.1	2.1	1.8	3.3

* 0.9 per cent sodium chloride perfusion begun.

† The appearance of fat droplets in this specimen indicated a general disintegration of the marrow.

Smears made from the sediment of specimens in this particular experiment showed but one megacaryocyte in the first oxalate specimen but several intact megacaryocytes and large numbers of naked nuclei of megacaryocytes in the saline specimens. This suggested that possibly the prothrombin found in our perfusion specimens came from the disintegration of megacaryocytes and not from the plates. The results of the oxalate perfusions fit in well with such an idea. It seems highly improbable that small elements like the plates if present in large numbers should not be washed out of the marrow by the oxalate solutions when myelocytes, leucocytes, and erythrocytes come out readily under such circumstances. The megacaryocytes on the other hand are large branching cells with processes extending into the marrow reticulum. It is reasonable to suppose that such cells would be fixed in place by an oxalate solution and could be broken up only by prolonged washing or by changing the perfusing fluid to some solution which did not act as a fixative. On the other hand initial perfusion with Ringer's solution or with 0.9 per cent sodium chloride might readily cause disintegration of cellular processes extending from the megacaryocytes into the vascular channels, and a consequent liberation of prothrombin into the perfusing liquid. This idea is advanced merely as a suggestive possibility.

(7) *Endothelial cells.* The possibility that the marrow prothrombin might arise from wandering endothelial cells was also considered. In investigating this possibility we stained one animal intravitally with trypan blue. One tibia of this animal was perfused with oxalate and the other with saline solution. The usual results were found as far as prothrombin was concerned. The oxalate specimens contained practically no prothrombin while the saline specimens were rich in this substance. Smears made from the sediments of the different specimens showed no intravitally stained cells in either the oxalate or the saline perfusates. Smears made after perfusion from the marrow tissue of both tibias, however, showed plenty of stained endothelial cells. From these observations one must conclude either (a) that wandering endothelial cells are not the source of the marrow prothrombin, or that (b) if they are a source of prothrombin they are neither disintegrated nor washed out of the marrow during perfusion but yield their prothrombin by a process of secretion. The former conclusion seems the more probable.

In summing up briefly the situation in regard to the cells of origin of the marrow prothrombin, we may say that there is no evidence that the prothrombin in question comes either from leucocytes, lymphocytes, myelocytes, nucleated red cells, or wandering endothelial cells. On the other hand the blood platelets are known to contain prothrombin, and the origin of the plates from the megacaryocytes of the marrow is well established. In the course of the work reported in this paper the authors have performed experiments in which bones perfused with 0.9 per cent sodium chloride or with Ringer's solution yielded large amounts of prothrombin while the sediment from such perfusion liquids contained practically no megacaryocytes. On the other hand bones perfused with sodium oxalate solutions yielded practically no prothrombin, but the perfusion liquids in these cases showed intact megacaryocytes and large numbers of naked nuclei of megacaryocytes. These facts all point to the megacaryocytes as a probable source of the marrow prothrombin.

CONCLUSIONS

(1) Evidence is presented that fibrinogen is not formed in the bone marrow.

(2) Prothrombin is formed in large amounts in the bone marrow.

(3) This prothrombin does not arise either from leucocytes, lymphocytes, myelocytes, nucleated red cells, or from wandering endothelial cells.

(4) The megacaryocytes are the probable source of the marrow prothrombin.

This work was undertaken at the suggestion of Dr. W. H. Howell, and we are greatly indebted to him for his constant interest and advice.

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EXPERIMENTS WITH OIL OF CHENOPODIUM AND CARDIAC STIMULANTS ON THE ISOLATED FROG HEART

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In a previous communication from this laboratory (1) it was shown that oil of chenopodium or ascaridol when injected intravenously into different animals caused depression of the circulation.

In continuation of these studies with the oil and its active principle, experiments were carried out on the isolated frog heart, special reference being had to antagonistic action for which purpose Adrenalin, Digitalis and Caffeine were employed. These drugs were perfused either in combination with oil of chenopodium or after the heart had been exposed to its action long enough to produce a pronounced effect.

The method is shown in figure 1. The oil of chenopodium or ascaridol, was shaken up with Ringer's solution and passed through filter paper, but later in the course of the investigation it was found advisable to add neutral olive oil to the perfusing fluid. This mixture was agitated for some time so as to make a fine suspension and was filtered before using.

When the fresh heart, soon after its excision, was perfused with oil of chenopodium, or ascaridol, in Ringer's solution no change in its action could be observed. In nearly every case the result of the first test proved negative, although the perfusion time as well as the amount of chenopodium added to the solution varied in many instances. The initial trial with 1 to 2 minims of ascaridol or oil of chenopodium in 100 cc. of Ringer's solution perfused for one-half to one minute failed to produce any change in cardiac activity. In some experiments, no signs of decreased action of the heart could be observed for a considerable time after its removal from the body, a number of perfusions with oil of chenopodium be-

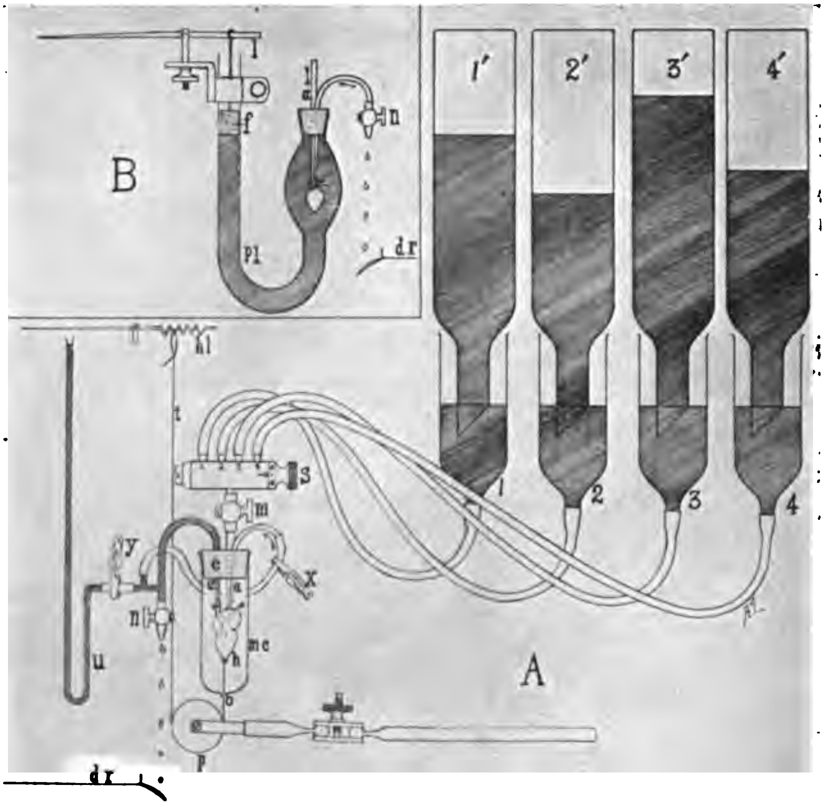

Fig. 1. A diagram of a modified form of frog heart perfusion apparatus described by Simpson (2) is shown in *A*. The double brass cannula (*a*) was inserted into the sinus and the glass cannula (*c*) into the aorta (heart *h*). Both cannulae are firmly fixed in a piece of cork (*e*) over which the moist chamber (*mc*) fits. Before the moist chamber is put in place a thread (*t*), fastened to the apex of the ventricle is passed through the opening (*o*) and around a pulley (*p*) to the short arm of a Harvard heart lever (*hl*). Perfusion from any one of the four reservoirs (*1*, *2*, *3*, *4*) may be made by turning the milled head of the Macmillan (3) stopcock (*s*) to the corresponding number. The four reservoirs (*1*, *2*, *3*, *4*) which automatically keep the fluids at the same level, together with the supply flasks (*1′*, *2′*, *3′*, *4′*) are held in a rack which may be vertically adjusted so as to provide any desired inflow *pressure*. The *rate* of inflow may be regulated by stopcock (*m*) and the outflow by stopcock (*n*). A Macmillan (4) drop recorder (*dr*) is used to register the outflow in drops. When it is desired to circulate the same small quantity of fluid continuously for some time stopcocks (*m*) and (*n*) are closed and clamps (*x*) and (*y*) are removed. The U-tube (*U*) allowed to fill to any height with the same fluid, shows the pressure against which the heart is working.

In some experiments the volume method was used as shown diagrammatically in *B*. In this case the double brass cannula (*a*), connected to the perfusion apparatus as in *A*, was inserted directly into the ventricle, one side serving as inflow and the other as outflow. The ventricle is immersed in the Ringer's fluid which completely fills the plethysmograph (*pl*). The variation in level of the fluid is transmitted by the float (*f*), to the recording lever (*l*).

ing carried out without causing any disturbance of function. As a rule, the fourth to the sixth perfusion caused depression, the period of exposure in each case being not longer than a half minute. Exceptionally, the effect may become manifest with the second perfusion. In one case, on the contrary, a very slight depression only was first noticed as a result of the twelfth trial. It may be pointed out in this connection that when the heart became susceptible to oil of chenopodium its effect generally increased with each successive perfusion until cardiac activity was temporarily (see fig. 2), and sometimes permanently abolished. The result seemed to be independent of the amount suspended in the perfusion fluid. One minim, and in some instances even a much smaller quantity, added to 100 cc. of Ringer's solution produced this effect (see fig. 2). It might further be stated that the action seemed to be independent

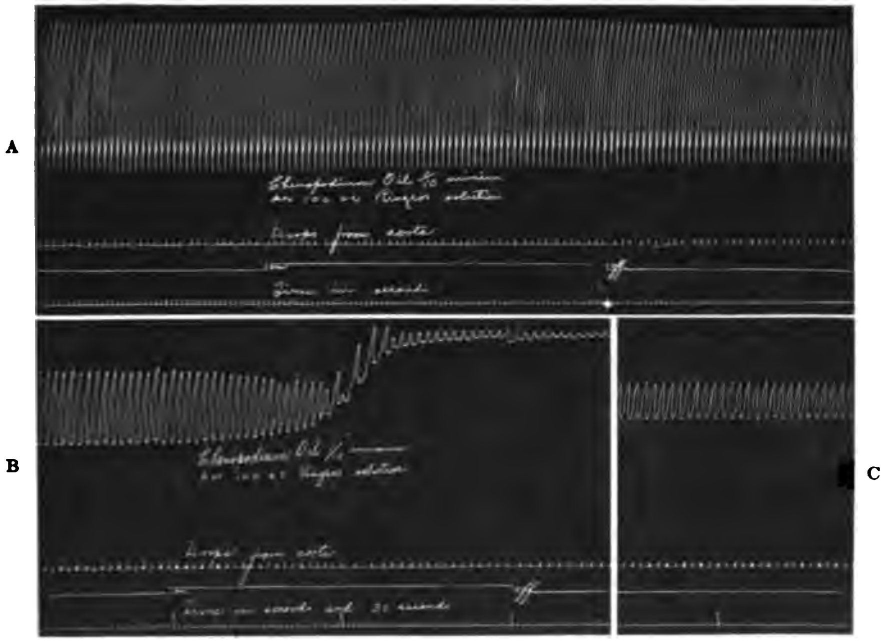

Fig. 2. *A*, Shows action of oil of chenopodium on the fresh heart. *B*, One hour after it had been perfused several times with oil of chenopodium. Heart in *diastole* (overdistended with fluid causing shortening of vertical diameter). *C*, Shows heart action twelve minutes after *B*.

also of the frequency and rate of perfusion, as the same was noticed after intervals varying between 3 and 12 minutes. This may be explained by the fact that oil of chenopodium is very sparingly miscible in water. It is quite possible that the saturation point was reached when very small amounts of oil of chenopodium were employed.

In order to make comparable tests with different amounts, a suspension of olive oil in Ringer's solution was resorted to as a suitable medium for dissolving the oil of chenopodium, thus enabling us to study the effect of concentration. One half cc. of neutral olive oil was added to 100 cc. of Ringer's solution and thoroughly shaken, then filtered and the desired amount of oil of chenopodium was added and again agitated. Control tests made to ascertain the action of olive oil showed at once that this was a very powerful stimulant whose action was particularly striking when the heart became weak as a result of prolonged perfusion with Ringer's solution. In some experiments the amplitude gradually increased until it reached three times its initial strength, moderate augmentation of rate being observed at the same time (fig. 3). The effect could be easily demonstrated by perfusing the heart alternately with Ringer's solution alone and with Ringer olive oil. Stimulation set in soon after the Ringer olive oil reached the heart and ceased when perfusion with Ringer's solution was resumed. The action of olive oil was observed about half a minute after perfusion was

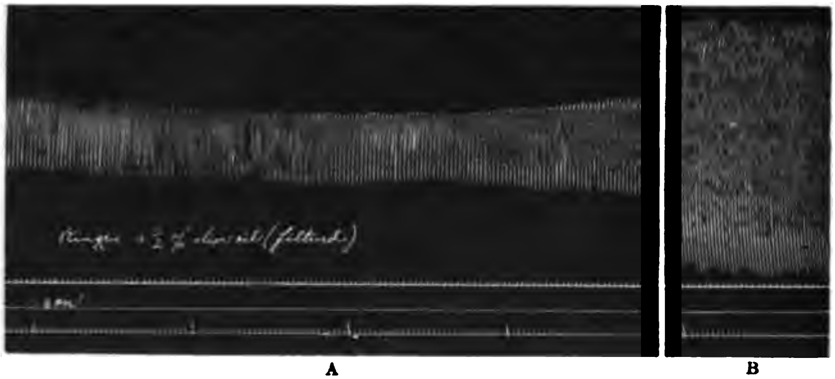

Fig. 3. Frog 109. *A*, General improvement of heart action when perfused with Ringer olive oil following perfusion with Ringer's solution. *B*, Effect of Ringer olive oil twelve minutes after *A*.

begun and remained uniform after reaching a maximum as long as perfusion with it was continued. It may be recalled that similar effects were obtained by Clark (5) with sodium oleate and other soaps of the higher fatty acids in the hypodynamic heart which is a condition of diminished activity established after it has been perfused for some time with Ringer's solution. When 3 minims of oil of chenopodium in 100 cc. Ringer's solution containing olive oil were perfused for 1 minute moderate depression, from which it soon recovered, was observed. Depression was sometimes produced,

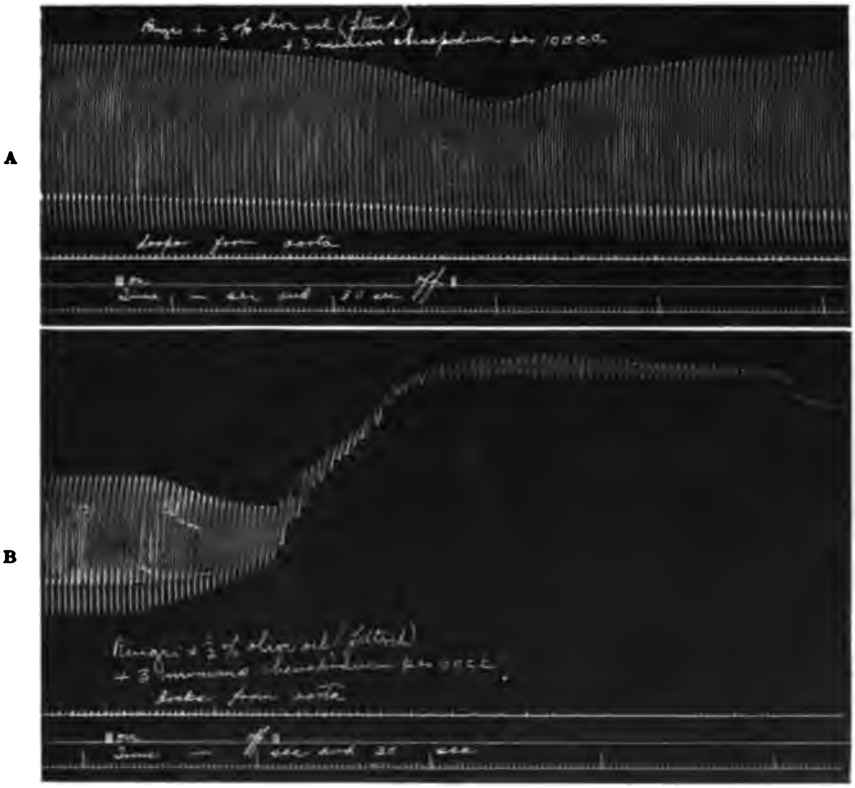

Fig. 4. Frog 119. *A*, First perfusion shows moderate effect of oil of chenopodium on the fresh heart. Perfusion time, one minute. *B*, Seventh perfusion. The same amount oil of chenopodium seventy-five minutes later, perfusion time about half. Heart in diastole (overdistended with fluid causing shortening of vertical diameter and apparent systole).

however, with one minim of oil of chenopodium to 100 cc. of R+olive oil. The action was more marked when the length of the perfusion time was increased. Thus, in two experiments in which 3 minims oil of chenopodium per 100 cc. were perfused 2 to 2½ minutes, heart action became very feeble in one case and almost disappeared in the other 1½ minutes after it was discontinued. In three other experiments in which perfusion lasted 3½, 4½ and 5 minutes, arrest of cardiac action was observed 3 minutes after the test with oil of chenopodium was begun. The effect was different with smaller amounts. Thus, whereas slight depression was produced with one minim to 100 cc. fluid when perfused for one minute, a decrease in rate varying between 60 and 70 per cent and a slight diminution of amplitude was noticed when the perfusion time was increased to 6 minutes.

The results with different amounts were very striking with the plethysmographic method of perfusion. One minim of oil of chenopodium to 100 cc. Ringer's solution and olive oil caused arrest of the heart in diastole for about 20 seconds; 1½ minims suspended in the same amount of fluid stopped the heart for more than 2 minutes, the length of perfusion time in each case being one minute. Although the dose and perfusion time are important elements in determining the action of oil of chenopodium on the isolated heart other factors, as already pointed out, must be taken into consideration.

The number of perfusions seemed to play an important rôle also in these experiments as the second or third perfusion with oil of chenopodium sometimes produced a much greater effect than the first. Temporary and sometimes permanent cessation of cardiac activity was observed after the seventh, eighth, or even after the sixth perfusion (figs. 4 and 5), 30 to 75 minutes after tests on the heart were begun. It is worthy of notice that the intervals between the tests varied between 5 and 47 minutes. That would indicate that the action of oil of chenopodium is cumulative. Evidence obtained in a number of experiments suggests, however, that the condition of the heart at the time oil of chenopodium is applied may be a strong causative factor in determining the action.

Thus the maximum effect was very often independent of the number and even of the frequency of perfusions with oil of chenopodium. In one experiment with 1½ minims to 100 cc. fluid the heart stopped after the third perfusion which lasted 1½ minutes, the intervals being 7 and 4 minutes. In another experiment with the same amount the heart stopped after it was perfused for 30 seconds, only one test

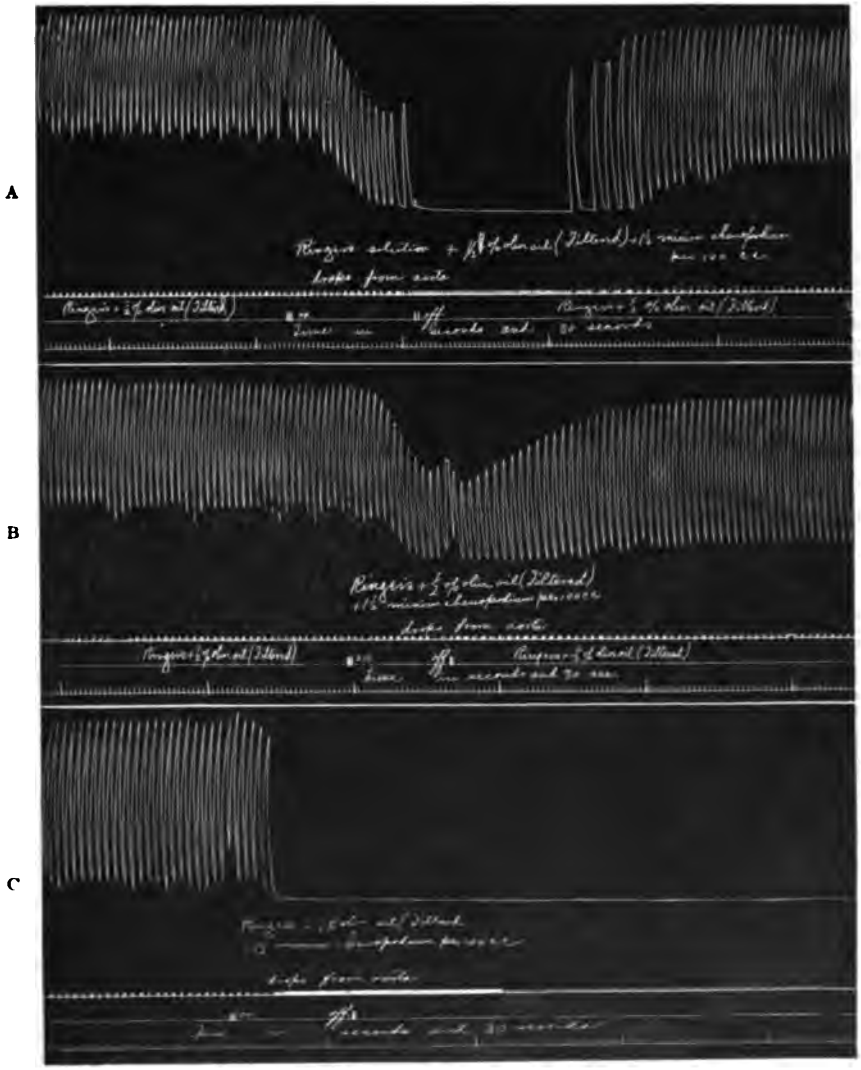

Fig. 5. Frog 85. Plethysmographic method. Ventricle only perfused. *A*, Effect of oil of chenopodium on fresh heart. *B*, Third perfusion, six minutes after *A*. *C*, Sixth perfusion, twenty-three minutes after *B*. Heart died.

with oil of chenopodium being made 25 minutes previously, the perfusion time in this instance being also 1½ minutes. In a third experiment the heart stopped with the first trial, the perfusion time being again 30 seconds. Only one minim oil of chenopodium was used. It may be observed that previous to perfusion with oil of chenopodium the heart had been perfused with olive oil in Ringer's solution for 15 minutes. A similar observation was made by Clark (6) in experiments with strophanthin. The reaction of the heart of the frog held in captivity showed a condition similar to that of the hypodynamic heart. That increased sensitiveness to oil of chenopodium may be compatible, however, with vigorous heart action was very strikingly illustrated in several experiments. In one case the contractions were uniform and vigorous immediately before the oil of chenopodium treatment, yet it proved to be very toxic, as the heart stopped almost immediately after the first trial with the drug which was tested after 15 minutes perfusion with olive oil. These variations in the resistance to oil of chenopodium were observed when the heart was perfused through the venous sinus as well as in experiments in which the plethysmographic method was used.

EXPERIMENTS WITH ADRENALIN

When the frog heart was perfused with Ringer's solution and olive oil containing oil of chenopodium and adrenalin, cardiac action in most experiments either remained normal or was distinctly stimulated, a marked increase in force of about 60 per cent and moderate augmentation of frequency being observed as a result of the treatment. The effect of adrenalin sometimes persisted several minutes after the perfusion. Occasionally, however, antagonistic action was absent, which may be accounted for by the rapid oxidation of adrenalin in oil of chenopodium, frequently observed in the course of our experiments. That it was not due to loss of potency of the preparation was shown by the reaction of the heart to adrenalin in Ringer's solution and olive oil without oil of chenopodium, as well as by blood pressure tests on rabbits. It might also be added that all the tests with oil of chenopodium which failed to respond to adrenalin were carried out in the early stages of the experiment. It is worthy of notice that the fresh heart was less sensitive to adrenalin as well as to oil of chenopodium than a heart which has been perfused for some time with Ringer's solution.

A series of experiments was also conducted, in which adrenalin was tried after oil of chenopodium in Ringer olive oil emulsion was perfused until heart action stopped or exhibited signs of considerable injury. The results established conclusively the antagonistic action of these two substances as shown in the following experiments. Condensed protocols are presented below.

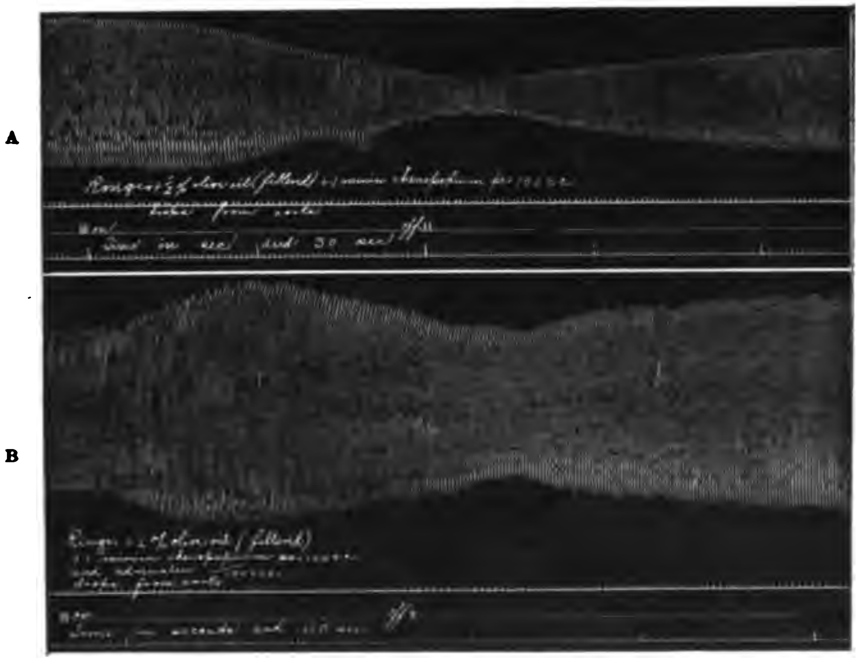

Fig. 6. Frog 103. *A*, Action of oil of chenopodium. *B*, Shows antagonistic action of oil of chenopodium and adrenalin.

Frog 102. One minim oil of chenopodium suspended in 100 cc. Ringer's solution and olive oil perfused for a half minute produced a moderate decrease of amplitude with some diminution of frequency from which it promptly recovered after the application was discontinued. Practically the same effect was observed 12 minutes later when such a solution containing 1: 100,000 adrenalin was perfused for an equal period of time. Adrenalin alone, however, produced the usual effect on the heart as was shown by a previous perfusion. Another perfusion with the same amount of oil of chenopodium for 25 seconds made four minutes later rapidly produced marked cardiac depression followed by pronounced irregularity of action. No improvement having occurred in 3½ minutes, perfusion with 1: 100,000 adrenalin in Ringer's solution and olive oil for one minute was resorted to. Within 20 seconds heart action became vigorous and regular, continuing in this condition for about 10 minutes.

Frog 169. The heart has been arrested in diastole, when perfused for one minute with 3 minims oil of chenopodium. A return of activity occurred almost immediately after the test with adrenalin was begun, the heart having been in a condition of paralysis 1¼ minutes. The contractions were at first feeble but they gradually increased.

Frog 170. Perfusion with 3 minims oil of chenopodium to 100 cc. Ringer's solution. Depression set in within two minutes, continued 3 minutes until the heart stopped. Perfusion with 1: 100,000 adrenalin made two minutes later had no effect. The experiment was repeated with a very strong solution of adrenalin 26 minutes after oil of chenopodium. Well marked and regular contractions appeared almost immediately and lasted 10 minutes. The heart continued to beat 15 minutes longer and died. The reviving action of adrenalin was also observed in another experiment.

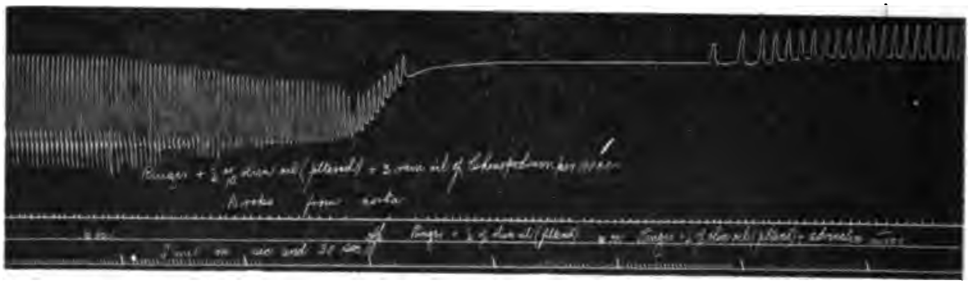

Fig. 7. Frog 169. Shows reviving action of adrenalin on heart arrested in diastole by oil of chenopodium.

Frog 172. In this case the heart was perfused with the same concentration of oil of chenopodium for 3¾ minutes. Cardiac action ceased in 2¾ minutes. A solution of 1: 100,000 adrenalin was perfused immediately after oil of chenopodium. Feeble contractions appeared within half a minute. Such prompt action was, however, not always obtained. That a longer interval may elapse between the application of adrenalin and return of cardiac activity is shown in the following experiment.

Frog 171. Heart perfused with 3 minims oil of chenopodium to 100 cc. fluid was arrested in diastole after 2½ minutes exposure to its action. Perfused 4½ minutes. One and one-half minutes later perfusion with 1: 25,000 adrenalin was begun and was continued 14 minutes Feeble contractions of the heart appeared within 3 minutes and steadily increased in force for 11 minutes until heart action was almost normal and remained in this condition. Several minutes after adrenalin was discontinued, heart action ceased but contractions reappeared when it was perfused again with adrenalin.

EXPERIMENTS WITH DIGITALIS

The official tinctures were used in these experiments, concentrations of 0.1 to 1 per cent being employed. The antagonistic action to oil of chenopodium was also observed with digitalis but the combined effect of the two drugs varied. Stimulation was observed in some cases, the effect in others being just sufficient to neutralize the action of oil of chenopodium, while in some experiments cardiac

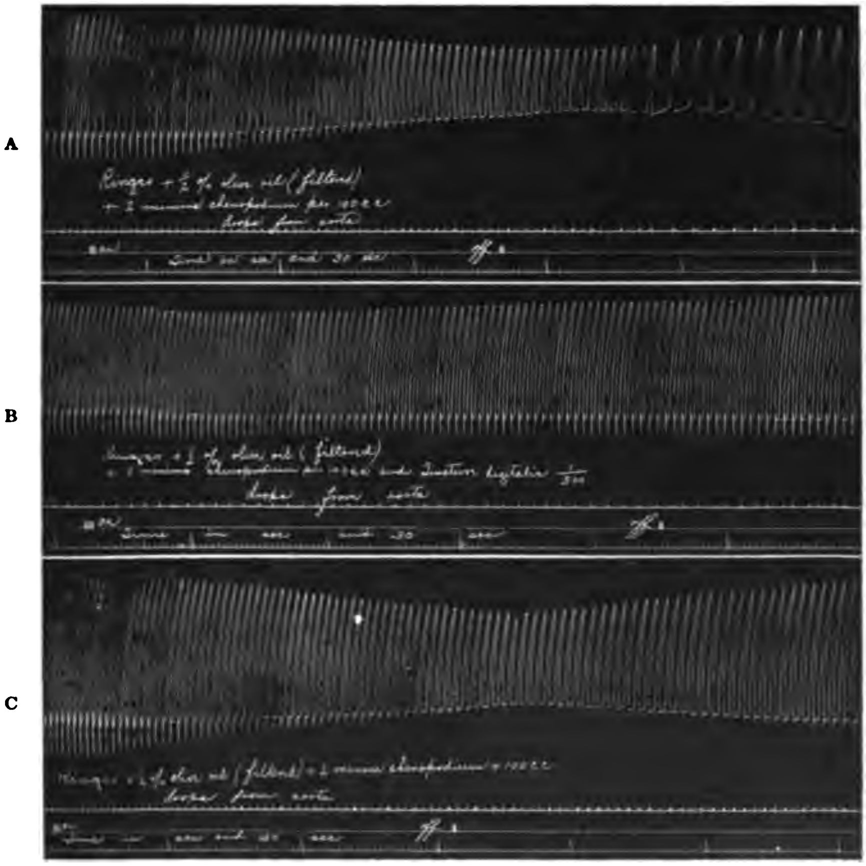

Fig. 8. *A*, The effect of oil of chenopodium. *B*, Antagonistic effect of digitalis and oil of chenopodium. *C*, Seventeen minutes after *B*. Previous treatment with digitalis decreases the action of oil of chenopodium.

depression, though slight, could be observed; in such cases a stimulating after effect lasting 5 to 7½ minutes occurred. It is worthy of notice that the effect of oil of chenopodium was less marked when preceded by treatment with digitalis. Thus in one experiment decreased action of oil of chenopodium was observed 17 minutes after it was perfused with digitalis, which may be explained by recent studies on the cumulative action of the digitalis bodies. Hatcher (7) has shown that the action of members of this group may persist for a considerable time, in some cases as long as 30 days. Corroborative evidence was furnished later by the experiments of Issekutz (8) in which the results indicated that the digitalis bodies form a combination with the substance of the heart which breaks up with difficulty. The antagonistic effect of digitalis was especially well shown in the following experiments: Two minims of oil of chenopodium in 100 cc. Ringer's solution containing olive oil were perfused for 1 to 1½ minutes. Marked cardiac depression was produced and was increased when the test was repeated 9 minutes later. The same amount of chenopodium in suspension in the presence of tincture of digitalis produced a slight primary depression followed by pronounced stimulation lasting in one test 5 minutes. That digitalis not only neutralizes the effect of oil of chenopodium but may cause stimulation was repeatedly shown in tests made on the same heart.

The antagonistic action of digitalis could be demonstrated much better when higher concentrations of it were used, though the amount of oil of chenopodium was increased. Thus in one experiment the heart was perfused 4½ minutes with Ringer's solution containing 1 per cent. tincture of digitalis and 3 minims oil of chenopodium per 100 cc. No change could be observed for 4 minutes. A slight decrease in amplitude without noticeable alteration in rhythm now appeared. Within 15 to 20 seconds, however, marked slowing of rhythm amounting to 50 per cent., and increase in amplitude occurred which, in several minutes, was 20 per cent greater than before oil of chenopodium was administered. Perfusion with the same amount of oil of chenopodium for 3¾ minutes carried out 24 minutes previously produced entirely different effects. After 3¼ minutes perfusion, the heart became markedly dilated, the contractions rapidly grew feeble until they almost disappeared. This condition lasted 3 minutes when the heart began to recover slowly, reaching a maximum in 6 minutes. Although the amplitude was now about the same as before administration of oil of chenopodium the rate was 35 per cent less. In another experiment a concentration of 1: 250 tincture digitalis was used for reviving the heart arrested in diastole by oil of chenopodium. Half a minute after perfusion with digitalis was commenced, contractions appeared. They were weak at first but rapidly increased in force though appreciably smaller than before oil of chenopodium was administered. The rate, however, was not changed.

The presence of alcohol in the tincture of digitalis may also be taken into consideration. A number of tests were carried out, therefore, with alcohol and oil of chenopodium in Ringer's solution and olive oil. One-eighth to one per cent of alcohol perfused one to two minutes failed to produce any change in heart action. Oil of chenopodium, one minim per 100 cc. was likewise ineffective. The combined action, however, of one minim oil of chenopodium and one per cent alcohol produced moderate depression. The depression which digitalis had to overcome is probably greater, therefore, than that produced by oil of chenopodium alone.

EXPERIMENTS WITH CAFFEIN

Although caffein, when perfused in combination with oil of chenopodium, produced moderate stimulation in the fresh heart, this was not the case when the same test was performed later in the experiment. No antagonistic action could then be observed. The effect indicated that caffein failed to stimulate the heart since depression occurred as in tests with oil of chenopodium alone. The results in some experiments suggested, on the contrary, that it aided the action of the oil of chenopodium. This was especially the case in experiments in which 0.01 per cent caffein was perfused with oil of chenopodium. In one experiment only moderate decrease of heart action was caused by chenopodium alone, but when perfused with a solution containing caffein the heart stopped in diastole for nearly one minute (fig. 9). Similar results were obtained in another case. Experiments with Ringer's solution with the same amount of oil of chenopodium in suspension and 1:500 caffein showed but little difference from those already described, although in one case in which oil of chenopodium was followed by 1:500 caffein, slight increase in heart activity was observed, amplitude alone being affected. That caffein may also cause depression was shown in another experiment in which the heart had been previously perfused with oil of chenopodium (fig. 10). It may be recalled that a similar observation was reported by Salant and Mitchell (9) on the isolated intestine, caffein likewise causing depression of smooth muscle previously subjected to the action of oil of chenopodium.

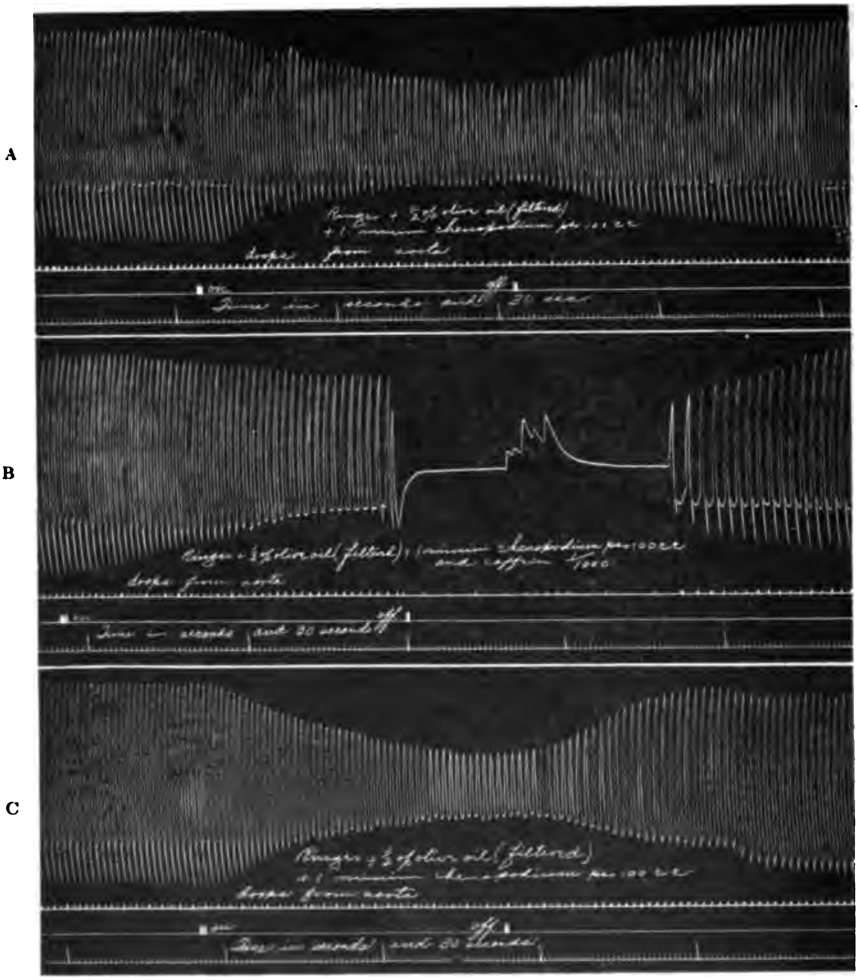

Fig. 9. *A*, Depression produced by oil of chenopodium. *B*, Combined effect of oil of chenopodium and 1/1,000 caffeine. *C*, The action of oil of chenopodium when perfused five minutes later.

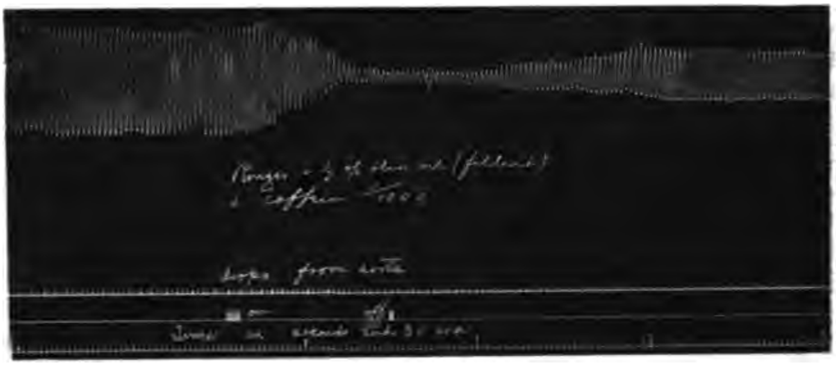

Fig. 10. Frog 94. Cardiac depression produced by caffeine after the heart had been perfused with oil of chenopodium.

DISCUSSION

Although small amounts of oil of chenopodium either failed to cause disturbance of cardiac action in the fresh heart or the effect produced was very moderate, the result obtained indicated that it was a depressant and varied directly with the perfusion time and the amount present in solution. The increased cardiac depression observed in successive perfusions suggested cumulation at first but later this was considered doubtful. It was found that in some experiments the effect was independent of the number of tests with oil of chenopodium. A heart which had been perfused for some time reacted to oil of chenopodium as well as one which had been subjected to its action a number of times, thus indicating that the heart is rendered more sensitive to oil of chenopodium the longer perfusion with Ringer's solution or Ringer olive oil is continued. The cause of this condition has not been determined, but experimental evidence brought forward by several writers shows that it might be due to loss of substances essential to the normal action of the heart. According to Schücking (10) cardiac action can only be maintained as long as traces of blood remain on the surface of the cells, and when this is washed out the heart can no longer continue to function. Herlitzka (11) found organic acids in Ringer's solution perfused through the rabbit's heart. Clark (12) claimed that there was a loss of lipoids from the frog heart when perfused with Ringer's solution. He separated the product from the perfused fluid and found that it exerted a marked beneficial action upon the hypodynamic heart similar to that previously obtained by Danilewsky (13) with lecithin. Jürss (14) has shown that thujone and fenchon dissolve lecithin. That it is also dissolved in oil of chenopodium was found in this laboratory. The lecithin and other lipoids of the heart might, therefore, be attacked by oil of chenopodium, thus producing the effect observed. The resistance of the fresh heart may also be thus explained. An abundant supply of lipoids in the intact animal may be assumed, the fresh heart containing a sufficient amount of them to stand the loss caused by the action of oil of chenopodium. As the store of lipoids is depleted by repeated perfusion, the action of oil of chenopodium would become more marked.

The increased effect of repeated perfusion and the difference in the behavior of the fresh heart and the heart after it has been perfused for some time may be due, therefore, to diminished amounts of lipoids present. The hypersensitiveness observed in some specimens may be similarly accounted for.

The mode of action of olive oil cannot be stated definitely. Inhibition of loss of lipoids by the oil or their accumulation at its surface causing cardiac stimulation as suggested by Clark (15) in the case of fatty acid is to be thought of as a possibility. But in view of the solubility of lipoids in olive oil, this explanation is not satisfactory. There would be no reason to expect the result to be different from that obtained with oil of chenopodium which is likewise soluble in lipoids. Its stimulating action on the heart probably depends upon other factors as yet unknown. The utilization of olive oil and the free fatty acids by the heart (thus replacing the lecithin which has been lost) is to be thought of in this connection. The formation of a protective envelope of colloidal particles around the cell, preventing the escape of lecithin, also suggests itself. The condition at the surface of the cell might become similar to that *in vitro* when lecithin, oils and soap are present. The stable emulsion formed would thus serve as a means of checking the loss of lipoids. The work of Lillie (16) is very suggestive in this connection. He has shown that the fatty acids penetrate the unfertilized egg of Arbacia and stimulate membrane formation and cleavage. As the same, in all probability, holds for the soaps, the cardiac stimulation observed with Ringer olive oil may be readily explained. The case is probably different with oil of chenopodium which resembles anesthetics in several respects. Thus, as already stated, we found that it aids the action of alcohol. Adrenalin restores cardiac action in paralysis caused by chloroform, chloral and oil of chenopodium. Lillie (17) observed that the action of neutral salts on the eggs of Arbacia is inhibited by anesthetics which he believes caused decreased permeability of the cell. Perhaps the action of oil of chenopodium is due to a similar mechanism. The antagonism of oil of chenopodium and olive oil may be thus explained, although both are lipoid soluble substances. The action of adrenalin and digitalis to oil of chenopodium may also be correlated in some way with the action of the cell lipoids. That they may form some combination with lecithin appears from the work of Koch (18). According to Schliomensun (19) digitalis combines with alcoholic phosphatids which he isolated from the human heart and from that of the dog. The antagonistic action of adrenalin and digitalis to oil of chenopodium is also of interest in showing that the heart when thus poisoned may be improved by substances differing in their mode of action, which indicates that the injurious effect probably involves the intercardiac nervous mechanism as well as the muscular substance. This is in harmony with our previous observations on the action of oil of chenopodium.

The action of caffein when perfused in combination with or after oil of chenopodium furnishes additional evidence that the action of the drug may be reversed under changed conditions, which was pointed out in a previous communication by the writer (20).

An explanation of the depressing effect of caffein observed by us is suggested by the recent work of Secher (21). He found that caffein hastens muscle fatigue and may cause histological changes in the finer structure of voluntary muscle fiber even when a dilute solution is used. He also observed that this effect is not peculiar to caffein as chloroform may produce the same structural alterations in muscle fiber. In view of the similarity of action between oil of chenopodium and anesthetics these observations are very significant. Perfusion with caffein would be more effective in producing the structural changes and the increased cardiac depression caused by oil of chenopodium. Again, since the injury caused by oil of chenopodium may persist, subsequent perfusion with caffein would simply further increase the damage already produced. This would also explain the depression observed after caffein when the heart had been previously subjected to the action of oil of chenopodium. The synergistic or additive effect of caffein and of oil of chenopodium on the heart which we also observed in experiments on the isolated intestine make it highly probable that oil of chenopodium attacks the muscle fiber. This supposition is strengthened by the antagonistic effect of digitalis and oil of chenopodium.

SUMMARY

1. Oil of chenopodium produces marked depression of the isolated frog heart, the effect increasing with the concentration and successive perfusions. The fresh heart is less sensitive to oil of chenopodium than one which has been perfused for some time.
2. Olive oil added to Ringer's solution causes cardiac stimulation which may become more marked later in the experiment.
3. Oil of chenopodium is less active in the presence of olive oil.
4. Adrenalin and digitalis were found to be antagonistic to oil of chenopodium.
5. Caffein produced slight stimulation when perfused with oil of chenopodium but in some experiments it aided cardiac depression.
6. The action of oil of chenopodium may be due to disturbance of the lipoids of the heart. It is suggested that the antagonistic action of olive oil, digitalis and adrenalin may also be correlated with disturbance of lipoid metabolism in the heart.
7. Oil of chenopodium probably affects the muscular substance in the frog heart.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

I. The Electrical Response as an Index of Glandular Action[1]

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In the extensive literature of electrophysiology much has been reported concerning observed alterations of electrical potential in nerves and muscles under various circumstances, but relatively little attention has been given to the electromotive phenomena of glands. The studies of du Bois-Reymond, Rosenthal, Hermann, Luchsinger, Engelmann, and Biedermann on the cutaneous and other surface glands in the frog and fish and in the cat, dog, pig and goat indicated, more than thirty years ago, the possibilities of interesting research in this field. To some extent these possibilities have been realized in studies of the psychogalvanic reflex. The use of electrical methods for examining the glands of the interior of the body, however, has been almost wholly neglected. In 1885, Bayliss and Bradford (1) reported the results of an investigation of the electrical changes attending secretion by the salivary glands in the dog and cat, and a few years later Bradford (2) contributed further information on the same subject. In 1894, Bohlan (3) published a paper on the electrical effects produced by the gastric mucosa when stimulated through the vagus nerves. Any other papers dealing with electromotive phenomena of the glands that lie distant from the body surface, we have been unable to find.

An interest in the activities of the ductless glands, especially those without a secretion recognizable by present methods, led us to consider the possibility of applying to them the extremely delicate means now available for detecting slight electrical differences, with the hope that thereby further knowledge of their functions might be secured.

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[1] The main results of this and the two following papers were presented before the American Physiological Society, December 29, 1915. See Proceedings, this journal, 1916, xl, p. 143.

Obviously an electrical change cannot yield information as to the nature of the materials elaborated in the several structures, it cannot determine the course which the products of secretion actually take in passing away from their region of origin, indeed it might leave somewhat uncertain whether secretion or preparation for secretion were the process occurring in a gland at any given period of disturbed potential. Nevertheless the method, by revealing the difference between relative quiet and activity, would indicate conditions which affect the functioning of glands and thus might suggest more definitive studies; it would show the quickness, the extent, and roughly the degree, of the response; and it might disclose significant new facts regarding relations which exist among the glands and which are mediated by the circulation. Despite its meager character such knowledge seemed worth seeking.

At the beginning of such a study of ductless glands there is obvious advantage in learning the capacities and limitations of the method as demonstrated in a gland which pours forth an easily recognizable secretion. For the purpose of preliminary tests we selected the submaxillary. And since we were, in the main, using cats in these researches we had the fortune of working on an animal in which both nerve supplies, the chorda and the sympathetic, evoke a considerable discharge of fluid. By use of the cat, therefore, it was possible to obtain insight into the electrical concomitants of a secretory activity which could be readily aroused from two different directions.

THE METHOD

The galvanometer used in these studies was of the d'Arsonval type.[2] Its sensitiveness is indicated by a deflection of 1 mm. on a scale 1 meter from the mirror when one volt is sent through 600 megohms. This sensitiveness, under the conditions of our observations, was sufficient for registering definite movements when physiological activity was induced in the glands, and yet was not so great as to make the instrument subject to minor adventitious alterations of potential.

The method of recording the galvanometer's deflection is shown by diagram in figure 1. On a firm wall-bracket, *T*, directly above the the scale, *S*, of the galvanometer, was placed a box painted black, inside and outside, and provided with a hinged cover, which when shut admitted light only through a horizontal slit, *O*, about 1 mm. wide. This slit was 11.5 cm. above the top of the scale. Inside the box was the drum of a horizontal kymograph; and since the mechanism of the kymograph was outside the box the rate of revolution of the drum could be readily adjusted. When a record was to be made a sheet of sensitized paper was wrapped tightly around the drum and fastened with gummed paper, the drum was then set on the spindle of the kymograph and lowered into the box, and the box closed. All this process was carried on while the room was illuminated solely with ruby light. The light reflected from the mirror, *M*, to the slit in the box had its source in *E*, an electric bulb (6 volts, 21 candlepower) the filament of which was held vertical and permitted to send its rays out to the middle of the mirror through a narrow vertical opening. The rays reflected from the mirror were focussed on the sensitized paper[3] by means of a thin lens, *L*, placed near the galvanometer. The distance from the mirror to the paper was 45 cm. Obviously any motion of the mirror around the vertical axis of its support would result in a shift of the reflected beam of light along the horizontal slit, *O*.

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[2] The instrument is that made by the Leeds and Northrup Company, Philadelphia, and listed as number 2220 in their catalogue.

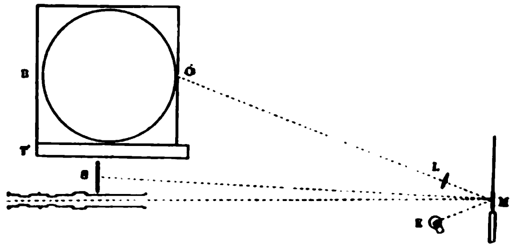

Fig. 1. Diagram of arrangement for observing and recording galvanometer deflections. For explanation, see text.

As indicated in figure 1, the arrangement allows the observer to watch the movement of the mirror through the telescope of the galvanometer at the same time that a record is being made.

A combined base line and time record was provided by a narrow beam of light directed on the slit, *O*, from another electric bulb. The electrical connections of this bulb are shown in diagram in figure 2. The current (at 8 volts) passing continuously through the filament of this bulb, *B*, was just sufficient to cause a slight glow which only dimly affected the sensitized paper. By means of another connection the dull glow of the filament was increased by an additional voltage, whenever a current was completed through the primary circuit of an inductorium *I*, by closure of the key, *M*. Thus the period of stimulation was registered on the revolving surface by a darkening of the dim base line. A clock, making an electric contact every 30 seconds, momentarily magnetized the iron core, *C*, and thereby drew the spring, *S*, away from contact 1, and against contact 2 (these contacts were platinum). The stronger current thus sent for an instant through the filament produced a sharp flash which was registered as a black point on the base line. This registering of the half-minute intervals interfered only for a fraction of a second with any stimulation that might be in process.[4]

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[3] The paper used was "standard" permanent bromide paper, quality B, made by the Eastman Kodak Company, Rochester, New York.

The non-polarizable electrodes used in contact with the tissues were similar to those described by Mines (4). Glass tubing, 8 cm. long and 5 mm. in internal diameter, was drawn to a point about 2 mm. in diameter. Into this pointed end was inserted a short length of soft cotton twine, which later afforded a gentle contact with the living tissue. The glass was then placed, point downward, in warm Ringer's solution containing 10 per cent gelatin. The fluid was deep enough to half fill the tubes. Six or eight such electrodes were thus prepared at one time. Usually this preparation was made in the afternoon for the next day. After standing over night in a cold room, the gelatin was so firm in the tubes that it supported in a thoroughly satisfactory manner the zinc sulphate poured in above it. When short zinc rods, soldered to copper wires[5] leading to the galvanometer, were placed in the zinc sulphate the electrodes were ready for use. These electrodes have each a resistance of approximately 1500 ohms. In the mass of gelatin in which they are set they can be kept for several days without deterioration, and removed as they are needed.

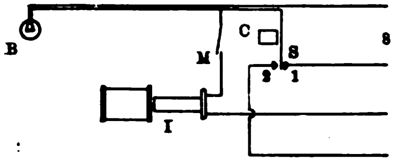

Fig. 2. Diagram of electrical connections for recording time intervals and duration of stimulation. For explanation see text.

Bayliss and Bradford, in their experiments on the submaxillary gland, set one leading-off electrode on the hilus of the gland and the other on the opposite surface. Apparently the object of this arrangement was to determine the difference of potential at the cell membranes lying between the duct and the lymph spaces. In our experience constant results have been obtained, however, with the gelatin electrodes situated one upon the gland being investigated, the other upon neighboring subcutaneous fascia[6] at a point near enough to other structures to be moistened with lymph. Secretion is then, except under conditions to be described later, accompanied by a negative relation of the gland to the indifferent surface, when these regions are connected through external conductors. Naturally the action current is occasioned only by such part of a circuit as is in fact active. And since stimulation of the chorda tympani, for example, makes active only the gland and not the surrounding structures, the current lines should be considered only with reference to the gland. The current lines would obviously be condensed under the electrode on the gland surface, but through the extensive area of contact between the gland and the tissues beneath it (which may be regarded as the other electrode) they would pass diffusely. In a complex gland like the submaxillary the highly confused relations of the cell membranes at which electrical differences would develop, and the simultaneity of action throughout the structure, would, in all probability, result in such antagonisms as to produce no effect whatever if only a sufficiently extensive region is taken for test. This suggestion is supported by the following experiment. We placed the two electrodes on outlying tissues on opposite sides of the gland and about 1 mm. distant from its border. Under these circumstances chorda stimulation failed to produce any action current that had an effect on the galvanometer; there appeared to be a nullification of the electrical differences in the secreting cells taken as a whole. An electrode placed at a point on the surface, however, should be regarded as having peculiar relations to the structures beneath. At that point doubtless there are cells facing ducts and backing on lymph spaces; and secretion by these cells would be accompanied by ionic alterations or by shifts of concentration, occurring at surfaces immediately below the electrode, which would definitely affect it. In other words, although a stimulated gland is simultaneously active in all parts, and consequently the attendant potential differences are likely to balance one another, still the electrical changes in a group of cells near the surface would be detected if one electrode is sharply localized in their neighborhood. These changes would produce current lines diverging from the electrode on the gland surface, passing widely-spread through the gland to surrounding tissues, and converging again to the electrode on the subcutaneous fascia. By this conception it has seemed to us possible to account for the point on the gland surface being negative to the indifferent point on the skin. But whether this explanation is true or not, the fact remains.

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[4] In developing the sensitive paper some sheets were darker than others. For uniform appearance the original photographs have been carefully copied in line drawings.

[5] The junction of the zinc and copper was covered with flexible wax.

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[6] Subcutaneous fascia was selected as an indifferent tissue because of Waller's testimony to its inactivity.

One of the two wires connected with the zinc rods previously mentioned was marked with a black band. When the rod attached to this wire was placed in the gelatin electrode on the tissue, and the other rod was in the electrode touching the gland, a *negative* relation of the gland to the tissue through the external connections would cause the galvanometer to register on the sensitive paper an excursion *upward* (positive) from the base line. A *positive* relation was registered by a movement of the light beam in the opposite (*downward*, negative) direction.

As soon as the electrodes were in place on the tissues and connected with the galvanometer they invariably revealed a difference of potential between the two points of contact. This difference had to be compensated before the gland changes could be conveniently observed. In figure 3 is shown diagrammatically the arrangement of apparatus for compensation. The source of the opposed potential was a Daniell cell, C. Wires from the cell were connected with a pole changer, *P*, which permitted the direction of the current through the rheocord, *R*, to be reversed at will. A resistance box, B, interposed in the circuit, made readily possible any desirable adjustment in the resistance. And by means of the slide, S, which moved freely along the exposed wire of the rheocord, the demarcation current from the gelatin electrodes, *E* 1 and 2, could be precisely opposed.

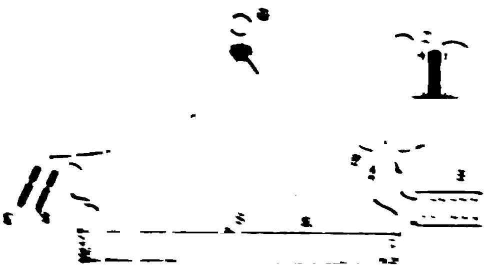

Fig. 3. Diagram of arrangement for connecting tissues with galvanometer, and for compensating the current of rest.

The presence of the compensating current, we found, became a source of error if the gelatin electrodes were both placed on the gland, or one on the gland and the other near it on adjoining tissue. Under these circumstances [illegible] of the gland by nervous impulses, for example, the galvanometer [illegible] the compensating current was [illegible] the galvanometer would move continuously [illegible] the compensating current [illegible] were lessened.

An action current may or may not be demonstrated with both electrodes on the gland. When it is developed, it is probably due to a difference in the relations of the underlying active tissue to the two points of contact. If, with both electrodes on the gland, the action current has the same effect as the compensating current, the galvanometer moves out sharply in response to the glandular activity (action current), and instead of returning to zero, as normally, after the action current, it continues slowly outward. And if the action current is opposed to the compensation current, stimulation sends the galvanometer outward as usual and there is a prompt return to zero, but now the galvanometer continues to move slowly beyond zero, i.e., as if now controlled by the compensating current. This phenomenon can be accounted for by assuming conditions which lessen the current of rest (increased conductivity, increased permeability of membranes?). It does not occur when a sufficient amount of tissue *outside the active gland* intervenes between the electrodes. Then the factor of lessened potential of the current of rest is minimized by the inclusion in the circuit of considerable undisturbed tissue which offers a resistance little affected by changes in the gland, and the action current alone is manifested on excitation.

In certain cases there was a tendency for the galvanometer to move continuously in one direction even though compensation was repeatedly effected. Usually this phenomenon was more marked when the gland was first exposed than later. The reason for the phenomenon has not been determined; though probably one factor was a greater evaporation at one pole than at the other. When present it was a source of difficulty, for, dependent on the direction of the tendency to move, the return of the galvanometer to zero after an excursion was either incomplete or excessive.

In order that the galvanometer may give as true a record as possible of the electrical changes due to physiological processes it is necessary, of course, to keep the parts under and near the electrodes as quiet as possible. While working in the neck region we found that an L-shaped cannula inserted into the trachea, and clamped so that it held the structures in the larnygeal region in some tension, reduced to a minimum the influence of respiratory movements. Occasionally, exposed muscles in the field of operation will twitch. This trouble can usually be obviated by cutting across the muscles, or by severing their nerves. The various other ways in which motion at the contact between the electrode and the tissue was eliminated in different circumstances will be described later.

In some of the earlier experiments anæsthesia was dispensed with after the brain and the spinal cord (as far as the mid-thorax) had been pithed. Although such a preparation is quiet and is free from the influence of any depressive narcotic, the blood pressure is possibly too low for normal action. After some experience with urethane and ether, we came to the conclusion that etherization, if not too profound, was the best mode of rendering the animal motionless.

THE POSSIBLE FACTORS IN THE ELECTRICAL CHANGES IN GLANDS

Stimulation of the nerve connected with a gland may have at least two direct effects, one on the gland cells, the other on the smooth muscle of the blood vessels; and it may have at least two indirect effects, an alteration of the rate of blood flow through the vessels, and the motion of secreted fluid along ducts. Conceivably any of these variations might produce a change of potential. Evidently if vascular changes, vasoconstriction or vasodilatation, were in themselves capable of giving rise to electrical differences, it would be difficult or impossible to draw conclusions regarding secretion in ductless glands having no demonstrable discharge. It is of prime importance, therefore, to determine what part any changes which are incidental to glandular activity itself may play in evoking electrical effects.

During the past five years evidence has appeared that contraction and relaxation of smooth muscle are accompanied by electromotive phenomena. The observations by Fuchs (6) on sipunculus and those of Brücke (7) on the retractor penis and of Orbeli and Brücke on the ureter have shown quite clearly that the electrical accompaniment of contraction is as characteristic of non-striate as it is of striate muscle. Still more pertinent was the observation by Straub (8), who noted, during the course of a study of the effects of certain conditions on the electrocardiogram, that the rise of blood pressure following asphyxia or a dose of adrenin was attended by a characteristic general shift of the galvanometer, which was recovered from as the pressure fell. He suggested that this phenomenon was a manifestation of the action current of the smooth muscle in the vessels. Although in Straub's experience local constriction of vessels, from exposure to cold, did not produce any electrical effect, and no shift was seen if the electrodes were on the same limb, the possibility of such an effect as a disturbing factor warrants its [illegible] out.

The question whether vasomotor changes might confound or modify the electrical phenomena of glands was discussed and tested by Bayliss and Bradford, and later especially by Bradford (9). They made use of the effect of atropine in abolishing secretion without disturbing the action of nervous impulses on the smooth muscle of the blood vessels. After injecting atropine they found that when the chorda tympani failed to produce a salivary flow from the submaxillary gland there was simultaneously a disappearance of the electrical phenomena. Since atropine does not interfere with the vasodilation due to chorda influences the conclusion was justified that this vascular change was not a factor in the galvanometric response. Confirmation of Bayliss and Bradford's claim is seen in figure 4, which records electrical changes in the submaxillary gland on chorda stimulation (*a*), and on sympathetic stimulation (*b*), before the administration of atropine (1 cc. per kilo of a 1 per cent solution,injected intramuscularly between *b* and *c*); and the failure of the changes on chorda stimulation (*c*), five minutes after the injection, and on sympathetic stimulation (*d*) with the same weak current that was employed at first. When the current strength was increased sympathetic stimulation was again effective (at *e*), but no response could then be evoked through the chorda tympani.

There is the possibility that, although relaxation of smooth muscle in the vessels of a gland does not disturb the galvanometer, contraction might do so. Of course a shortening of the vascular muscle occurs during recovery from chorda dilation, and yet, as shown above (figure 4, *c*) no deviation of the galvanometer appears. The sympathetic influence on the vessels of the gland (in the dog) affords evidence, however which is more positive. Atropine in a sufficiently large dose not only inhibits the influence of the chorda tympani but also that of the sympathetic impulses. And Bradford found that when such a dose is given to a dog the abolition of the secretory effect of the sympathetic is coincident with the abolition of the electrical phenomena. Still other evidence that vasoconstriction alone, in the absence of secretion is not effective in producing electrical differences is found in the results of injecting pituitrin. As is well known, pituitrin causes a general rise of blood pressure by a direct constrictor action on the arterial walls. We have injected pituitrin[7] (0.2 cc.) into the femoral vein of a cat and raised the general blood pressure from 102 mm. of mercury to 158; there was, however, no secretion, and the galvanometer showed no electrical change whatever.

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[7] We used the fluid preparation made by Parke, Davis and Company, Detroit.

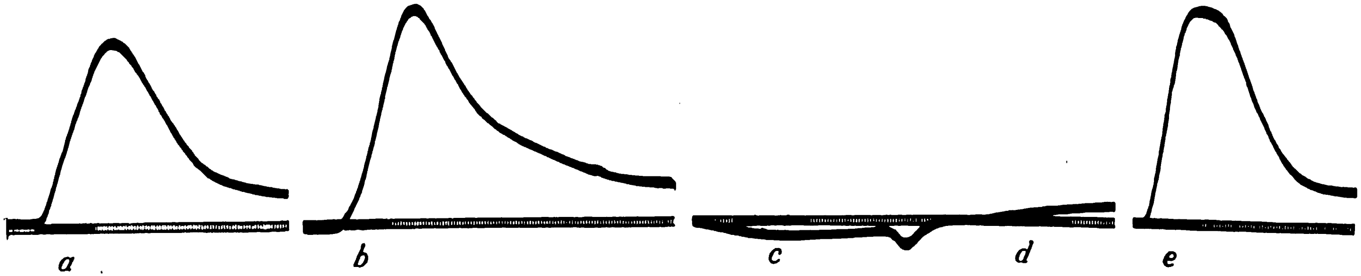

Fig. 4. Abolition of typical electrical response of submaxillary gland by atropine. Upper record, galvanometer; lower record, periods of stimulation; at *a* chorda stimulation 10 seconds, at *b* sympathetic stimulation 10 seconds, between *b* and *c* atropine 1 per cent, 1 cc. per k. injected intramuscularly, at *c* (5 minutes after the injection) chorda stimulation, at *d* sympathetic stimulation, at *e* sympathetic stimulation with much stronger current, which was ineffective when applied to the chorda. Time not recorded.

From these various experiments the conclusion seems well warranted that the electrical phenomena accompanying secretion are not due either to contraction or to relaxation of the smooth muscle in the vessels of the gland.

Another condition concomitant with vasomotor changes and also with external secretion is an altered rate of flow of fluid through capillaries and ducts. It has long been recognized that the movement of fluid through fine tubes is capable of causing differences of potential (10). May not the faster or slower blood flow and the progress of secreted material occasion the electrical phenomena?

Local vasodilation would be accompanied by a faster streaming, local vasoconstriction by a slower streaming through the capillaries. As already noted, both vasodilation and vasoconstriction will occur after atropine has stopped secretion of the submaxillary gland. But when secretion is lacking the electrical effect also is lacking. Under these circumstances, therefore, the altered rate of flow cannot have any influence in creating a difference of potential. We have tested the possibility further by raising blood pressure in the head through sudden occlusion of the aorta below the diaphragm and by suddenly lowering the pressure through compression of the heart. A pressure of 120 mm. of mercury was thus raised to 182 mm. and later lowered to 66 mm. Neither the faster flow from the high pressure nor the slower flow from the low pressure had any effect on the galvanometer. An altered rate of blood flow, we may conclude, is not a factor in producing the electrical change.

Although, in our experience, differences in the speed of streaming blood had no electrical effects, complete stoppage of the stream usually produced a deflection of the galvanometer which varied in extent and promptness of appearance. It seems probable, because of these variations, that the electrical changes are not consequent on the physical changes in the blood flow but are due rather to the influence of anemia on the gland cells.

Evidence indicates that the movement of secreted material along minute ducts of a gland likewise does not condition the electrical response. Bradford (11) noted, and we have confirmed him (see figure 5), that the galvanometer deflection starts before the saliva begins to move, and the maximum rate of the deflection is reached before the maximum rate of flow from the duct. Furthermore, when the escape of saliva is prevented by compressing the duct, repeated stimulations cause the usual electrical effect, though, as might be surmised from the development of a back-pressure, the effect becomes gradually less (see figure 6, *b*, *c*, *d*). If now by removal of the compression the accumulated saliva is allowed to flow freely no potential difference appears (see figure 6 *e*).[8] This absence of an electrical change as secreted fluid begins to move or ceases to move along ducts is in accord with the conclusion reached in the previous paragraphs that the physical changes in the blood flow—starting, stopping, or going faster or slower—are not the occasion for electromotive phenomena.[9]

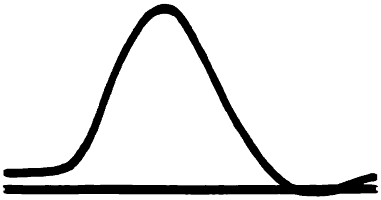

Fig. 5. Electrical change starting before saliva begins to move. Upper record, galvanometer. Lower record, dark band, chorda stimulation, 10 seconds; vertical lines, drops of saliva, except first one which marks moment when saliva began to flow in cannula. Last drop, white line on curve. Time not recorded.

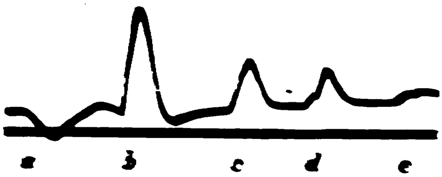

Fig. 6. Action currents of submaxillary gland after duct closed. Upper record, galvanometer. Lower record, time in half-minutes; at *a* clip on duct (manipulation); at *b*, *c*, and *d*, chorda stimulation, 10 seconds; at *e* clip removed (manipulation).

An action current will accompany secretion after the blood supply has been removed.[10] Figure 7 records the electrical changes occurring in the right submaxillary gland. At *a* the right common carotid artery was clamped (the left had been clamped previously). About one minute later (at *b*) the cervical sympathetic was stimulated for ten seconds. The usual electrical change occurred attended by secretion.

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[8] It was impossible to avoid slight electrical changes due to manipulation while the clip was being applied and removed (*a* and *e*).

[9] In this discussion the reservation is made that a more sensitive galvanometer might reveal electrical differences not shown by the instrument used by us.

At *c* the clamp was removed from the right carotid. The record of electrical change differs from the normal chiefly in the absence of a rapid return to the base line (cf. figures 5 and 6), but the upward curve is characteristic and adds further to the proof that the electrical response does not arise from vascular changes. Favorable to that conclusion, also, is Bohlan's observation that vagus stimulation calls forth an action current of the gastric mucosa even though the aorta is clamped.

Other reasons for concluding that the functional activity of the secreting cells, and not muscular contraction or movement of fluid through fine tubules, determines the appearance of an action current in glands, will be noted in later papers. Thus the latent period of the action current in the thyroid gland differs from that of the adrenal, and in both these glands the period is much longer than in the submaxillary. Also the duration of the action current differs markedly in different glands; in the submaxillary it may last not more than a half-minute, in the thyroid and adrenal it commonly lasts much longer, as a result of brief electrical stimulation of nerves. There is no reason for supposing that the smooth muscle in the blood vessels of these glands varies either in the quickness of its response or in the persistence of its altered state (contraction or relaxation). The variations in the character of the action currents, typical of the several glands, contribute strongly to the argument that the electrical changes are reliable manifestations of a functional response.

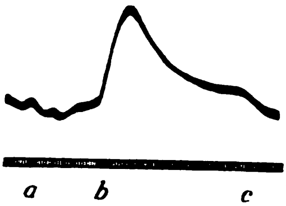

Fig. 7. Action current of submaxillary gland after carotid arteries closed. Upper record, galvanometer, connected with right submaxillary gland. Lower record, time in half-minutes; at *a* right carotid closed (left closed previously), at *b* sympathetic stimulated 10 seconds, at *c* clip removed from right carotid.

SUMMATION PHENOMENA

As illustrating the possibility of using the electrical method to obtain insight into the conditions of activity of glands the results of combined chemical and nervous stimulation may be cited. Figure 8 shows the usual effect of nervous influences after pilocarpine. At *a* 1 cc. of pilocarpine (1:1000) was injected into the femoral vein. A persistent secretion soon started, accompanied by a persistent positive electrical change. At *b* the chorda tympani was stimulated for 15 seconds, but without effect. About 30 seconds later (at *c*) stimulation of the cervical sympathetic trunk sent the galvanometer deflection from +40 to +35, then out to +42, and slowly back to +25.[10] In this instance the excitation induced by pilocarpine was such that the chorda influences, which pilocarpine is supposed to mimic, no longer had any effect. The sympathetic influences, however, were still capable of increasing the activity of the gland.

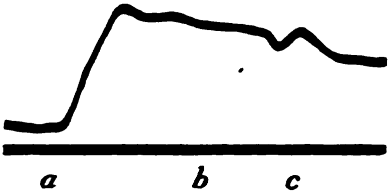

Fig. 8. Ineffectiveness of chorda impulses and effectiveness of sympathetic impulses during pilocarpine action. Upper record, galvanometer. Lower record, time in half-minutes; at *a* 1 cc. pilocarpine (1: 1000) injected intravenously, at *b* chorda stimulation, at *c* sympathetic stimulation (see text).

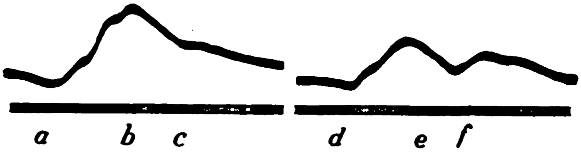

Fig. 9. Effectiveness of chorda impulses and ineffectiveness of sympathetic impulses during adrenalin action. Upper record, galvanometer. Lower record time in half-minutes; at *a* 1 cc. adrenalin (1: 10,000) injected intravenously, at *b* chorda stimulation, at *c* sympathetic stimulation, at *d* another adrenalin injection (1 cc., 1: 10,000), at *e* sympathetic stimulation, at *f* chorda stimulation.

The conditions of excitation in the submaxillary are quite different when adrenin is administered. In the cat this substance calls forth a secretion of watery saliva and an attendant electrical effect. In figure 9 is shown the record of the galvanometer after 1 cc. of adrenalin (1: 10,000) was injected (at *a*) intravenously. When the effect was maximal, the chorda tympani nerve was stimulated (at *b*), and the movement in the positive direction was definitely increased; at *d* another injection of adrenalin was made, and near the summit of the curve stimulation of the sympathetic was without influence, but when on the return the chorda was stimulated a considerable increase of the positive deflection was registered (from +5 to +12). In another instance, after sympathetic impulses had been proved impotent during the acme of adrenalin action, a second dose of adrenalin caused an excursion to +26; and when at this maximum, chorda stimulation for 10 seconds increased it to +41. In these instances excitation of certain glandular components by adrenin made ineffective the sympathetic impulses which this substance simulates; but other components subject to chorda influences were not involved, and when they were stimulated, the gland, as the galvanometer shows, became still more active.

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[10] The plus sign (+) indicates a movement of the galvanometer so that record above the base line is registered, i.e., the gland is negative to the fascia (see p. 44). The numbers refer to millimeter divisions on the galvanometer scale.

Confirmation of these results was obtained by stimulating for five seconds, first the chorda tympani, and then, after the galvanometer had moved to a maximum of +22, stimulating for the same period the sympathetic trunk. The excursion was thus increased to +32. Conversely, when sympathetic stimulation preceded and the deflection had reached +20, excitation of the chorda fibres increased it to +42.

REVERSAL OF THE SIGN OF THE RESPONSE

A phenomenon which has been repeatedly noted in electrical studies of glandular action is reversal of the response. Thus Engelmann (12) reported that the usual effect of stimulating the nerve of a piece of frog's skin is a negative variation of the normal "in-going" (i.e., external surface negative to internal through the galvanometer) current of the skin. In Hermann's observations (13), on the other hand, the commonest effect was a positive variation—an increase—of the normal current. Bayliss and Bradford (14), although admitting that it is "scarcely possible to speak of a normal excitatory variation," found, by making examinations throughout the year, that most of the time they confirmed Hermann but during the breeding season (February and March) they saw what Engelmann had seen. According to Waller (15) the reversal of response may occur within a few minutes—the first effect of stimulation is in the Engelmann sense, the second, five minutes later, is less so, and the third, five minutes later still, is in the direction Hermann described.

We have had similar experiences in recording the action currents of internal glands. For example in one animal the thyroid gland when stimulated will be negative in relation to the indifferent skin, in another it will show regularly a positive relation.[11] This is admittedly disturbing. Various suggestions come to mind as possibly accounting for it, such as the position of the electrode on the gland, the degree of pressure, a reversal of activity, the lessening of an activity already existent rather than its increase on stimulation, or so much greater permeability that the compensating current prevails. Many of these suggestions were ruled out by a result which we obtained in some animals on stimulating either the chorda tympani or the cervical sympathetic trunk after the submaxillary gland was set in prolonged secretion by pilocarpine (1 cc. 1:1000, intravenously). Under these circumstances the nerve impulses, instead of causing the gland to become increasingly negative to the skin, rendered it less negative (see figure 10, *c*, *d* and *e*). And when the potential accompanying the secretory activity was compensated, sympathetic or chorda stimulation (figure 10 *f*) instead of making the gland negative to the skin (as figure 10, *a*) now made it positive. Later the nerve impulses caused a primary slight movement of the galvanometer in the "normal" direction, but there followed at once a much more extensive excursion opposed to that. In these experiments there was no shift in the position of the electrode nor any variation of its pressure on the gland. As a result of the stimulation there was the normal effect of chorda impulses—a marked increase in the rate of secretion. Lessening of the physiological process or reversal of it cannot, therefore, be invoked to account for the phenomenon.

For the present the reversal of the sign of the response remains unexplained. It may belong in the same category with numerous other instances of exactly opposed effects of the same stimulus, dependent on the state of the responding tissue (16). From the evidence here given, however, we may rest assured that the electrical change, either positive or negative, which follows stimulation is an indication of glandular activity. Like other indicators of fundamental physiological processes the electrical change develops gradually to a maximum and more gradually subsides again, and it follows after a definite latent period. As Waller has remarked, the obvious delay between excitation and response is "a sure and reassuring sign that we are dealing with a true physiological response, outside all possibility of coarse physical fallacy."

It is obvious that many more aspects of the functioning of the submaxillary gland are capable of investigation by the electrical method.

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[11] The terms "negative" and "positive" we employ with the understanding that they refer to two regions as related through external connections.

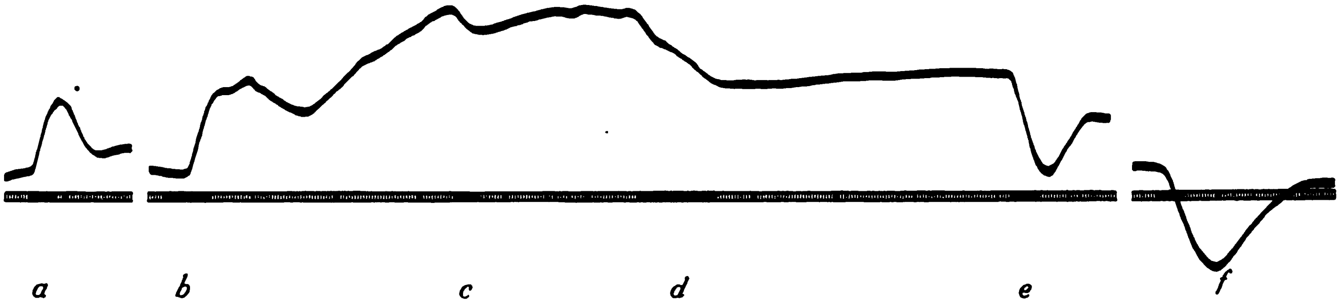

Fig. 10. Reversal of electrical response during pilocarpine effects. Upper record, galvanometer. Lower record, time in half-minutes; at *a* chorda stimulation, at *b* 1 cc. pilocarpine (1: 1000) injected, at *c* sympathetic stimulation, at *d* sympathetic stimulation again, at *e* chorda stimulation (with return to zero), at *f* chorda stimulation again after compensation. Secretion continued during the whole period after *b*, and was increased during the nerve stimulations.

For the present, however, they may be left. The brief study here reported was of value to us chiefly for justifying the method as a means of getting more knowledge of the conditions of activity of ductless glands. We believe that the evidence here presented warrants the employment of this method in such an investigation and that when a difference of electrical potential appears in a gland as a result of an adequate stimulus the electrical effect is substantial evidence of secretory activity.

SUMMARY

A method is described for registering the electrical changes of a [illegible] gland [illegible] while permitting [illegible] [illegible] of the [illegible] [illegible] the [illegible].

Because (1) an electrical change accompanies secretion of saliva [illegible] [illegible] the [illegible] is [illegible] from the gland or the flow [illegible] the duct is [illegible], and because (2) the change is absent when secretion is absent [illegible] it is shown that the electrical change is a manifestation solely [illegible] [illegible] [illegible].

[illegible] [illegible] [illegible] [illegible] [illegible] [illegible] a [illegible] activity of the [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] the electrical [illegible] is strongly [illegible].

[illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] gland may [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] remains [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] in the [illegible].

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[illegible]

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCERINE GLANDS

II. THE SECRETORY INNERVATION OF THE THYROID GLAND

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"One of the most difficult and yet one of the most important problems of modern physiology is the question of the innervation of the glands of internal secretion." With this sentence Osokin begins a recent paper on the thyroid gland. Among our chief objects in undertaking this series of studies on the electrical phenomena of glands was to throw as much light as possible into the obscure regions of our acquaintance with the innervation of the ductless glands. Because of an interest which one of us had in another research on the thyroid we selected this gland for our first examination.

Histological evidence. That the thyroid is well supplied with nerves has been known for many decades, as a result of histological studies. There has been general agreement among investigators that these nerves have not been distributed solely to blood vessels, but come into relation besides with the peculiar glandular cells of the organ. As far back as 1867, Parameschko (1) found nerves leaving the vessels and passing away as varicosed branches in the connective tissue between the follicles. In 1875 Poincaré (2) made similar observations, and intimated that these fibres might have a secretory function. And since then plexuses of nerve fibres about the follicles and connected with the bases of cells have been reported by Crisafulli (3), by Andersson (4), and by Berkeley (5). Andersson's description has recently been confirmed by Rhinehart (6). According to this latest work all nerves entering the thyroid gland are non-medullated; they arise from neighboring cervical sympathetic ganglia, accompany the arteries, and follow their branches after entering the gland. From the perivascular plexuses fibres are given off to interfollicular connective tissue and form there other plexuses completely surrounding the follicles. From each perifollicular plexus the finest fibrils reach out and end in knob-like enlargements on the basal end of the gland cells.

From the analogy of other glands provided with secretory nerves, we should expect that the cells of the thyroid would be supplied solely by fibres from the autonomic nervous system. The histological evidence, just cited, that only non-medullated fibres enter the gland, gives confirmation to that surmise. These fibres, however, might be derived from both the sympathetic and the cranial divisions of the autonomic system, or from either division alone. It is a matter of considerable significance for understanding the relation of the thyroid to the bodily economy to learn their origin.

Anatomical evidence. After careful anatomical and embryological dissections Briau (7) reported that the thyroid nerves arise from the cervical sympathetic trunk, leaving that trunk at different levels, but originating especially (in man) from the middle cervical ganglion. This mass of nerve cells usually lies upon, or close to, the inferior thyroid artery, hence the name "thyroid ganglion" assigned to it by Haller. It corresponds to the inferior cervical ganglion of the dog and cat. Even what in man has been described as the inferior cervical ganglion is frequently found fused with the first thoracic. It is this fusion of several ganglionic masses at the top of the thorax that makes the stellate.

In man the fibres passing out from the middle (inferior) cervical ganglion form plexuses around the thyroid arteries, Briau states, but chiefly around the inferior artery. According to Poirier and Charpy (8) and other anatomists, branches from the superior cervical ganglion make the superior thyroid plexus about the artery of that name, and penetrate the gland along with it. Henle's observation that the superior laryngeal nerve, and Lindemann's observation that the recurrent laryngeal each sends two or three filaments to the gland, were confirmed by Briau in all his dissections.

In the cat the inferior cervical ganglion lies low in the neck, and the superior cervical is situated well above the level of the thyroid cartilage. The inferior thyroid artery along which in man filaments from the inferior ganglion pass to the gland is inconstant in the cat, and even when present is small. The superior thyroid artery is a regular branch of the common carotid. Langley states (9) that occasionally in the cat a small "accessory" ganglion is found on the cervical sympathetic trunk, about the level of the superior thyroid artery, which sends off gray fibres to that artery. In most animals this ganglion is part of the superior cervical.

Evidence from stimulation or severance of nerves. Further indication of the character and relations of these nerves is found in the experimental work done to determine whether or not they have a secretory function. Evidence has been obtained by stimulating the nerves or severing them, and testing the effects on other functions or on the condition of the gland itself.

In the Bern laboratory Asher and his collaborators have used the method of stimulation and examination of other functions. They have followed a suggestion made by Cyon (10) that the superior and recurrent laryngeals are the secretory nerves for the thyroid. When these nerves were excited Asher and Flack (11) found that the depressor causes a greater fall of arterial pressure and that adrenin raises arterial pressure to a greater height than before. Because the same phenomena occur after intravenous injection of thyroid extract they concluded that the laryngeal impulses call forth a true secretion. Asher and v. Rodt (12) reported that stimulation of the laryngeals increases the efficacy of splanchnic impulses. And Oswald (13) observed that such stimulation magnifies the influence of the vagus on the heart. Although Biedl and [illegible] (14) were able to demonstrate that the depressor causes a greater fall of pressure after the laryngeal nerves have been excited than before, they were unwilling to concede that this result was a reliable indicator of thyroid secretion, because in the same experiment induction shocks applied to the depressor resulted in a variable fall. And Schäfer has testified (15) that he has been unable to confirm, either in the rabbit, the dog, or the cat, the claim that injection of thyroid extract increases the efficacy of the cardiac vagus or the depressor. In any case, whether Asher's criterion of thyroid discharge is trustworthy or not, the method leaves unanswered the interesting question whether the impulses are delivered by cranial autonomic or sympathetic neurones. For although the laryngeals are branches of the vagus and therefore contain preganglionic elements of the cranial division, they might, on the other hand, serve merely as conductors of sympathetic fibres.

[illegible] more direct evidence as to the [illegible] of [illegible] in the [illegible] is found in tests of the iodine content of the gland with and without stimulation. [illegible] by [illegible] (16), by [illegible] and [illegible] (17), and by [illegible] (18) agree in showing that the iodine [illegible] per gram of gland substance, though [illegible] in [illegible] the two lobes of [illegible]. [illegible] and [illegible] that [illegible]-

lation of the superior thyroid vessels with the accompanying nerves, or the vago-sympathetic trunk (in the dog), may make the iodine content of the disturbed lobe less than that on the other side by as much as 0.3588 mgm. per gram of gland substance. They concluded, therefore, that a discharge of the peculiar substance of the thyroid takes place in response to nervous stimulation. This conclusion has been supported by Watts (19) who, after stimulating the cervical sympathetic trunk, or the nerve filaments accompanying the superior thyroid artery (in the dog), found a considerable reduction of the iodine content of the thyroid on the stimulated side. After stimulation of the entire sympathetic trunk the average reduction (0.04572 per cent for dry tissue) was more than it was when the thyroid filaments alone were excited (0.03526 per cent).

Additional evidence indicative of sympathetic influence over thyroid activity was obtained by Missiroli and by Wiener. Missiroli (20) found at various periods after section of the sympathetic trunk in the neck characteristic changes in the granules of the secreting cells of the thyroid. Wiener (21) studied the effects of cutting not only the sympathetic, but also other nerves. He severed the vagus on one side (in the dog) above the ganglion nodosum, but without effect—the glands on the two sides corresponded in weight and in iodine content just as in normal animals. The result was quite different after removing the inferior cervical ganglion; within a few weeks a remarkable diminution in size occurred on the operated side (in three cases a lessening of dry weight of 36.9, 48.5, and 11 per cent),[1] and with that a corresponding reduction of the thyroglobulin content. Katzenstein's earlier observation (22) that severance of the superior laryngeal nerve results in a degeneration of cells in the gland, Wiener explains as a consequence of section of sympathetic fibres in the superior laryngeal, because cutting the main trunk of the vagus is without effect.

He concluded that if paralysis of the sympathetic causes atrophy and a lessened iodine content, stimulation must cause hypertrophy and increase of thyroglobulin—a conclusion which is opposed, however, by the results of the stimulation experiments already described.

The various interpretations which have been given to the experiments above sketched will be left to later discussion. For the present it is sufficient to state, in summary, that the evidences from histological and anatomical examination, and from experimental excitation and severance of nerves, point towards a definite influence of sympathetic impulses over thyroid activity.

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[1] In a cat with the thoracic sympathetic cord cut just below the stellate ganglion we found after four months the thyroid lobe on that side weighing 0.167 gram, that on the other side 0.256 gram.

Electrical evidence for the innervation of the thyroid gland.[2] In a previous paper (23) we have given reasons for believing that the action current of a gland is a reliable indication of secretory activity. The two phenomena, as tested in a gland with external secretion, appear together, vary together in duration, disappear together, and are independent of accompanying vascular disturbances. This close concomitance of secretion and electrical change justifies the use of the electromotive phenomena of a gland to determine the conditions of its activity.

Methods. The general methods which we have employed in this research we have described in the first paper of this series. We found in studying the thyroid gland that urethane (2 grams per kilo) was unsatisfactory as an anaesthetic, and also that deep etherization was capable of abolishing effects that appeared readily when the anaesthesia was not so profound. In some experiments we obtained consistent results on dispensing with any anaesthetic after pithing the brain and the cord to the mid thorax. Since the blood flow was thus much diminished, however, we decided that etherization to the point of immobilizing the animal was a less upsetting procedure, and consequently we have employed that method in most of our work. In a few cases we have used curare after decerebration.

Through an opening between the second and third ribs the right sympathetic trunk was laid bare. Shielded electrodes which could be fixed in place[3] were slipped under the nerves just below the stellate ganglion, and the conductivity of the fibres below the electrodes was destroyed by crushing. The skin was now drawn close around the projecting part of the electrodes. Artificial respiration was maintained by an interrupted air current. The leading off (gelatin) electrodes, connected with the galvanometer, were set, one in contact with the right thyroid gland which had been exposed with as little manipulation as possible, the other on the connective tissue inside of a little flap of skin turned back from the neck at about the thyroid level, or at times on the trachea. Movement of the parts near these electrodes, due to respiration, was practically abolished by clamping the tracheal cannula and pulling it slightly towards the chest. Usually just after the gland was exposed and the gelatin electrodes were applied a period of repeated adjustment had to be passed through before the tissues and the compensating current were in equilibrium.

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[2] The brief description of experiments which follows gives no indication of the troubles which were encountered in the course of our investigations. The method was new to us, it was new in its application to glands of internal secretion, and was beset not only with experimental difficulties, but also with dangers of serious error. We lost much time before we learned that deep anaesthesia would check secretion. We had difficulties in securing immobility and excluding the effects of action in neighboring tissues. We also had troubles with electrodes and with diffuse action of chemical stimuli. All these and other conditions we were compelled to examine for their influence on the processes being studied and on the methods which we were using. After we had learned the pitfalls, however, and could intelligently avoid them, we found it possible to demonstrate in one preparation practically all the points here described.

[3] See Cannon and Nice: This journal, 1913, xxxii, p. 47. A small spear-shaped steel hook reaching out of the wood held the rod in any position desired.

Effects of sympathetic stimulation. Numerous observations have shown that when the sympathetic trunk immediately below the stellate ganglion is stimulated by a weak tetanizing current a definite difference of potential is developed between the two leading-off electrodes. This phenomenon does not occur if the stimulus is applied to other nerves in the upper thorax, e.g., the central end of the cut phrenic. It is not, therefore, due to any spreading of direct physical influences from the thorax to the upper neck region. Furthermore it does not occur if the leading-off electrode on the gland is transferred to a neighboring muscle, and the thoracic trunk is then stimulated. It is, therefore, due to some change in the thyroid itself. That the electrical change has a characteristic and long latent period—usually between 5 and 7 seconds after the stimulus is started—which is considerably longer than that for the submaxillary gland, confirms the other evidence that the change is not direct physical effect, but a peculiar and distinguishing manifestation of thyroid activity.

In the first paper of this series, on the submaxillary gland, records were published illustrating the gland negative to the skin (through external connections) as a result of nerve stimulation, and also, but under different circumstances, the gland positive to the skin as a result of the same stimulation. The reversal of skin currents, also, in consequence of repeated nervous excitations during a short period, was referred to. Reversal of the current is not yet explained, but that it does not invalidate the electrical phenomenon as a criterion of activity is proved by the observation that a typically increased flow of saliva follows chorda stimulation, whether the gland becomes negative or positive to the skin (24). These facts are recited here because, in our experience, the thyroid gland, although usually negative to the skin after stimulation, is sometimes positive for a moment and then negative. More rarely a purely positive relation to the skin is present.

Illustrations of these three types of responses are given in figure 1, A and B, and in figure 2. Other records taken before and after these in the same animals had the same peculiarities. The latent period in the case of figure 1 B was longer than in the case of A, a condition which was noted in several other instances in which the reaction occurred in

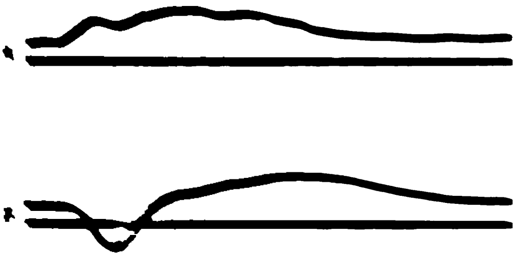
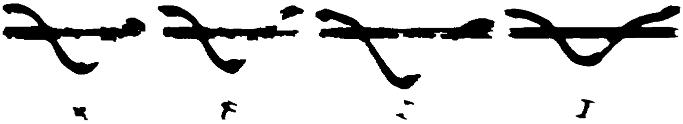


Fig. 1. [illegible] of stimulating thoracic sympathetic [illegible] time is registered in half-minutes. A, stimulation [illegible] minute, giving negative [illegible]. B, stimulation a half minute, giving first positive, then negative.



[illegible] half-minute [illegible] B [illegible]

[illegible]

[illegible]

was stimulated (at 2.39) with all the cervical nerves intact; and figure 2 *B*, a record from the same animal (at 2.43) after the recurrent laryngeal was cut The conditions of stimulation were the same in both instances. The response was that of a positive relation of the gland to the indifferent tracheal cartilage. The operation has evidently not affected the influence of impulses started in the sympathetic fibres of the chest.

The action current of the thyroid persists also after the influence of the superior laryngeal nerve has been abolished. In figure 2 *C* the action current of the thyroid is registered on stimulation of the sympathetic trunk in the thorax (at 3.09) after severance of the superior in addition to the recurrent laryngeal. The stimulus was applied for a half-minute just as before; the larger response is probably not significant of a favorable effect of the operation, because, as proved by other instances in which the same experiment was performed, the response after the nerve cutting may be slightly less than before.

A further step in determining the path of the impulses which cause the electrical change is taken when the cervical sympathetic fibres are sectioned, and the thoracic trunk is stimulated. Figure 2 *D* is a photograph of the electrical change of the thyroid in the same case from which the other records in figure 2 (*A*, *B* and *C*) were taken, but after the cervical sympathetic trunk in addition to the laryngeal nerves had been cut across. Obviously these operations have not interfered with the reaction of the gland. Although the reaction continues after the sympathetic fibres have been transected in the neck, excitation of these fibres central to the cut produces the characteristic effect.

The question now arises as to where the relay between preganglionic and postganglionic fibres is situated. As already seen, two ganglionic masses are present in the course of the cat's cervical sympathetic—the inferior and the superior, with occasionally an "accessory" mass near the superior thyroid artery. That all the fibres leading to the gland do not originate in the superior ganglion or ganglia is proved by figure 2 *D*, for in that instance the sympathetic trunk had been cut across between the two cervical ganglia. It can be assumed therefore that in the cat a relay of some of the fibres occurs in the inferior ganglion.

Evidence for a relay in the superior region was obtained by the nicotine method of blocking impulses at sympathetic synapses and also by serial sectioning of the nerve from above downwards. In one of the experiments in which nicotine was used stimulation of the cervical sympathetic sent the galvanometer from +2 to −3, and thence out to +94. A pledget of cotton wet with 1 per cent nicotine was now laid in contact with the superior cervical ganglion. Eighteen minutes later the nictitating membrane was not withdrawn when the sympathetic trunk was stimulated, but the galvanometer was sent out to +76; eight minutes later another stimulation for a half-minute sent it to +78. Now a slender strand of cotton wet with the nicotine solution was laid along the sympathetic trunk from the superior cervical ganglion to a point 2 cm., below the thyroid cartilage. After 20 minutes, stimulation of the cervical trunk had no effect. That this was not due to any deleterious effect of nicotine in the gland was proved by exciting the gland to activity by chemical stimulation (in a manner to be described later), and also by applying the electrodes to fibres on the superior thyroid artery and finding that repeated stimulation sent the galvanometer in the plus direction. In another experiment in which the sympathetic was serially cut, first across the upper pole of the superior cervical ganglion, second across the middle, and then crushed directly below the ganglion, stimulation of the cervical sympathetic trunk still persisted in causing marked action currents in the thyroid gland. The sympathetic trunk was now severed about 1 cm. below the ganglion, and thereupon the electrical effect of stimulation disappeared. These experiments indicate that in these animals the upper relay region was not situated in the superior cervical ganglion itself, for when impulses to the eye had been blocked in that ganglion by nicotine or the fibres had been cut below it, the electrical change in the thyroid still followed excitation of the sympathetic. The impulses were shut off, however, when the nerve was cut some distance below the ganglion or when nicotine was allowed to act on that region, i.e., the region in which Langley found the "accessory" ganglionic cells with a distribution of fibres to the thyroid. In another instance, after nicotine, applied to the superior cervical ganglion, had failed to prevent the thyroid action current (although effectively blocking nervous influence on the eye), a strong carbolic acid solution touched on the superior thyroid artery, in order to destroy nervous conductivity, quickly abolished the phenomenon.

After the cervical sympathetic trunk has been cut above the inferior cervical ganglion and a record of the thyroid action current has been obtained by stimulating the thoracic sympathetic cord (as in figure 2, *D*), excision of the inferior cervical ganglion abolishes the effects of stimulation.

From the foregoing evidence we may conclude that both in the superior and in the inferior ganglionic masses of the cervical sympathetic, impulses to the thyroid gland pass from preganglionic fibres to the outlying neurones.

The question of vagus control. The prominence of laryngeal stimulation in previous experiments, especially the experiments carried on in the Bern Laboratory, raises the question as to whether the thyroid gland like the submaxillary of the cat, is subject to control by cranial as well as by sympathetic autonomic fibres. To secure evidence on this question, we had to stimulate the vagus trunk before its union with the cervical sympathetic strand. And since the vagus contains motor fibres to muscles of the larynx, which might through their contractions affect the galvanometer, it was necessary to use a curarized preparation. We decerebrated, and then, in order to avoid as far as possible the danger of disturbing the gland cells, injected intravenously barely enough curare to abolish reflexes. We bared the vagus at its point of emergence from the skull, tied a thread about it there, cut proximal to the thread and applied the stimulus to the nerve as close as possible to the thread, i.e., as far as possible from the union of the vagus and sympathetic at the superior cervical ganglion. We have not succeeded in obtaining any evidence of any influence of vagus impulses on the thyroid gland. We have stimulated the nerve with the gland at rest, and with the action current developing as a result of sympathetic impulses, and with the action current disappearing—but no change was produced. Figure 3 *A* is a record of the action current of the right thyroid as a result of stimulating for a half-minute the right thoracic sympathetic cord (at *a*). After a latent period of about 25 seconds (longer than with ether—probably due to curare and a lowered body temperature), the galvanometer began to indicate the gland negative to the indifferent tissue. When the galvanometer had moved to +12, the right vagus trunk was stimulated for a half-minute with the same stimulus (at *b*). The movement continued without check to +45, and was followed by a slow return. That vagus stimulation had no noteworthy influence, is shown by comparing figure 3 *A* with figure 3 *B*, a record taken three minutes after figure 3 *A*, and registering the

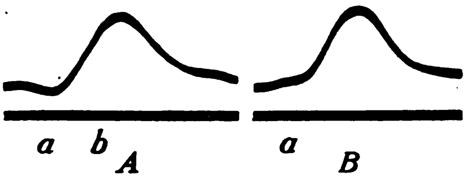


Fig. 3. *A*, 4.27. Thyroid action current on stimulating for a half-minute the thoracic cord at *a*, and the main vagus trunk at *b*. *B*, 4.31. The same, on stimulating the thoracic cord alone at *a*.

action current when the thoracic cord alone was excited for a half-minute (at *a*). The close resemblance of the two records proves that the vagus has been neither an exciter nor an inhibitor of thyroid activity.

From this evidence we may conclude that the laryngeal branches of the vagus nerve are not secretory nerves of the thyroid gland. And the fact that action currents of undiminished amplitude follow sympathetic stimulation after these nerves are severed (see figure 2) indicates that they are not highly important vehicles for sympathetic fibres.

Pharmacological evidence. A way of securing further evidence bearing on the source of impulses affecting the thyroid is found in the use of drugs having specific effects on cranial autonomic nerve endings. It is well known that pilocarpine stimulates viscera innervated by cranial autonomic nerves and affects these organs in such manner as to mimic the action of nerve impulses. In the case of the submaxillary gland, for instance, chorda impulses are commonly ineffective if the gland is already responding to an injection of pilocarpine (25). By use of pilocarpine, therefore, it is possible to obtain deeper insight into the nature of the nerve supply to the thyroid.

Figure 4 shows the lack of effect on the thyroid gland when 1 cc. of pilocarpine, 1: 1000, was injected (at *a*) intravenously. This is a dose which in the cat produces an abundant and long-continued salivary secretion, accompanied, as shown in the first paper of this series, by a persistent electrical change. But in the thyroid gland pilocarpine does not induce an action current—under no circumstances have we observed consistent indications of an effect on the thyroid which could be ascribed to this drug. These results are in accord with the failure of vagus impulses to influence thyroid activity; they support the conclusions from the histological studies by Schmid (26) and by Osokin (27), and the chemical studies by Wiener (28) that pilocarpine is wholly without effect on the thyroid; and they are further evidence against the opinions of Wyss (29) and of Andersson (30), based on microscopic appearances, that pilocarpine produces changes in the thyroid similar to those seen in a secreting gland. The weight of evidence, therefore, is opposed to a cranial autonomic supply to the thyroid gland.

The influence of anemia on thyroid activity. In a recent paper Watts (31) has reported that diminishing by mechanical means the blood supply to one thyroid lobe, periodically (for 2 seconds every 10) during approximately three hours, lessens the iodine content of that lobe relative to the other. The discrepancy between the two lobes was not so great as when a stimulus was applied to the cervical sympathetic or to the fibres on the superior thyroid artery, but such stimulation, it was pointed out, would cause a greater check to the blood flow than could be caused by mechanical compression of the arteries. Watts and Carlson conclude, therefore, that shutting down of the blood supply by vascular constriction accounts for all changes in the thyroid thus far shown to be caused by stimulation of nerves.

We have put to test the idea that anemia accounts for thyroid secretion. In figures 1 *B*, 2 and 3 the period of stimulation was only a half-minute. Even if the result were a total stoppage of the flow through the gland, it would not persist after stimulation ceased. That complete anemia of the thyroid for a half-minute is practically without effect is shown in figure 5. For two periods, at *a* and at *b*, the blood supply to the gland was entirely excluded. This was done by first tying the left carotid artery low in the neck, and then, at *a* and at *b*, occluding the right carotid. It was impossible to avoid a slight amount of disturbance from manipulation, which would account for the uneven line of record. The effect of this procedure is to be compared with the effect of stimulating for a half-minute the thoracic sympathetic cord in the same animal, at *c*. Obviously nerve stimulation has an influence on the thyroid very different from anemia.

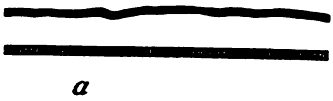


Fig. 4. Absence of thyroid action current on injecting pilocarpine (1 cc., 1:1000) at *a*.

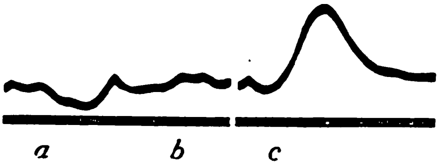


Fig. 5. Galvanometric changes in the same animal when thyroid completely anemic at *a* and at *b* (a half-minute each) and when stimulated (thoracic cord) a half-minute, at *c*.

Discussion of the evidence for thyroid innervation. The observations presented in the foregoing pages are concerned mainly with three questions: (1) the nature of the innervation of the thyroid gland, (2) the mode of action of the nerve impulses whether directly secretory or not, and (3) the character of the process underlying the electrical change.

We believe that the evidence is now convincing that the nerves of the thyroid are exclusively derived from the sympathetic division of the autonomic system. The histologists find non-medullated fibres reaching to the base of the follicular cells; the anatomists distinguish only cervical sympathetic nerves entering the gland; sympathetic section causes marked atrophy of the gland structure whereas vagus section has no influence; sympathetic stimulation gives rise to a striking action current, whereas excitation of the main vagus trunk, and exhibition of pilocarpine as a cranial autonomic stimulant are without effect. All these facts are harmonious in bearing testimony to an exclusive sympathetic control of thyroid activity. The observations on the effects of exciting the laryngeal nerves, made by other investigators, are not thereby rendered invalid, but the interpretation placed upon them is that whatever influence these nerves may have on thyroid function is exerted by way of sympathetic fibres contained in them—a possibility which, as we have seen (p. 59), has anatomical justification.

The question as to whether the nerves supplied to the thyroid are truly secretory in function has been raised for various reasons. Although regarding the sympathetic as the trophic and secretory nerve of the thyroid, Wiener has argued (32) that if section of the sympathetic produces atrophy of the thyroid, stimulation must produce hypertrophy, and accumulation of thyroglobulin. This conclusion, however, is not justified. Section of the chorda tympani, for example, results in a gradual lessening of the size of the submaxillary gland (33), and yet the normal function of the nerve is to cause a secretory discharge. Also Wiener's argument is opposed by the discovery that stimulation of the cervical sympathetic trunk actually lessens the iodine content of the gland. The view of Watts and Carlson that sympathetic impulses lessen the iodine content by producing anemia fails to regard both the direct nerve supply to the gland cells and also the reasonable assumption that these fibres have a function apart from the fibres distributed to blood vessels; and furthermore their view is opposed by the evidence presented in this paper that although sympathetic stimulation induces a marked action current in the thyroid, total anemia for an equivalent period brings forth no noteworthy electrical change whatever. Prolonged anemia may indeed lessen the iodine content of the thyroid, but tissues respond to a variety of stimuli, and merely because, for example, anemia causes convulsions, it is not proper to attribute all convulsions to uremia. The conclusions of Watts and Carlson, therefore, seems to us defective in failing to regard important conditions which affect response. Still another doubt regarding secretory fibres has recently been raised by Manley and Marine (34) who found that when thyroid tissue is transplanted to various parts of the body it grows where placed, and functions and reacts as a normal gland would do. There is no logical opposition to secretory nerves in these experiments. The adrenal glands, for example, will serve the routine needs of the body when their secretory nerves are cut, for the animal lives with the nerves transected, but he promptly dies if the adrenals are removed. The capacity of an organ to give service, as the heart may do, though separated from the central nervous system, should not lead to the conclusion that its nerves are without influence. This doubt of the existence of specific secretory nerves to the thyroid has not, therefore, any convincing significance. And like the other suspicions as to the reality of these nerves, this one is based, as we see it, on a failure to consider all the evidence.

A part of the evidence for secretory nerves is, to be sure, first presented in this paper. But it is based on the assumption that the action current is a sign of secretory activity—and *that* might be doubted. To some degree the assumption is based on analogy with the submaxillary gland. An action current in that gland is the regular concomitant of secretion, and fails when secretion fails. The action current of the thyroid has a latent period characteristic of physiological processes, it may outlast the period of stimulation by many minutes, it appears after stimulation of sympathetic fibres—the filaments of which are known to be distributed to the gland cells—and all these evidences indicate that the action current is a true signal of physiological events taking place in the gland as a result of nerve stimulation. The concordant testimony of different investigators that precisely the sort of stimulation which provokes this process, will, if continued, result in a measurable diminution of the iodine content of the disturbed lobe affords clear proof that the process signalled by the galvanometer is one of secretory discharge.

SUMMARY

Histologists describe non-medullated nerve fibrils reaching to the cells of the thyroid gland. Anatomists find that fibres going to the thyroid gland arise in cervical sympathetic ganglia.

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(15) Schäfer: The Endocrine Organs, New York, 1916, 36.
(16) Wiener: Arch. f. exper. Path. und Pharm., 1909, lxi, 297.
(17) Rahe, Rogers, Fawcett and Beebe: This Journal, 1914, xxxiv, 72.
(18) Watts: This Journal, 1915, xxxviii, 363.
(19) Watts: Loc. cit., 360.
(20) Missiroli: Arch. d. Fisiol., 1909, vi, 588.
(21) Wiener: Loc. cit., 310.
(22) Katzenstein: Arch. f. Physiol., 1897, 371.
(23) See Cannon and Cattell: This Journal, 1916, xli, 39.
(24) See Cannon and Cattell: Loc. cit., 54.
(25) See Cannon and Cattell: Loc. cit., 52.
(26) Schmid: Arch. f. mikr. Anat., 1896, xlvii, 181.
(27) Osokin: Russkiy Vrach, 1915, xiv, 300.
(28) Wiener: Loc. cit., 309.
(29) Wyss: Korrespondenzblatt f. Schweizer Arzte, 1889, xix, 178.
(30) Andersson: Loc. cit., 201.
(31) Watts: Loc. cit., 367.
(32) Wiener: Loc. cit., 313.
(33) Bernard: Journ. d. l'Anat. et Physiol., 1864, i, 507.
(34) Manley and Marine: Proc. of the Soc. for Exper. Biol. and Med., 1915, xii, 202 .

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

III. The Influence of the Adrenal Secretion on the Thyroid

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One of the most interesting possibilities offered by the use of the action current as a sign of glandular functioning is that of testing assumed interrelations among the ductless glands. Of course, the effect of activity of one gland on the activity of another would have to be accomplished with reasonable rapidity in order to be demonstrated by the galvanometer. It is quite possible that the influence of some glands on others, such, hypothetically, as that of the ovary on the thyroid, might be so gradual in its development as not to be recognizable by an electrical change. The conditions might be different in other relations in which quick effects are produced. Thus the adrenal glands are known to respond promptly to stimulation, and their secretion is rapidly influential. The thyroid glands also are capable of ready action, as shown by the brief latent period of their response after sympathetic stimulation. Consequently if the adrenals are capable of exciting the thyroid bodies, the effects should be demonstrable by means of the galvanometer.

Because in their experiments injection of adrenin increased the basal protein metabolism in dogs—a condition the same as that seen in hyperthyroidism and the opposite of that produced by removal of the thyroid glands—Eppinger, Falta and Rudinger (1) declared that the adrenal medulla probably exerts a stimulating influence on the thyroid gland. Since stimulation of sympathetic fibers induces in the thyroid gland a marked electrical change, as the paper before this one demonstrates, and since adrenin everywhere in the body duplicates the action of sympathetic impulses, the suggestion that adrenin and adrenal secretion may stimulate thyroid secretion seems highly reasonable. It is a suggestion easily put to test.

Figure 1 is the record made by the action current of the thyroid when 0.1 cc. of adrenalin (1: 100,000)[1] was injected (at *a*) intravenously. After a latent period (which in different instances varied from about 10 seconds upwards) the galvanometer was sent out to +77 and at the end of five minutes had slowly returned to +7, when the drum was stopped. This result and many others like it, have proved clearly that adrenin has a striking effect on the thyroid gland.

In a previous paper we have presented evidence that the nerve impulses delivered to the thyroid by sympathetic fibers do not act by lessening the blood supply, but stimulate the gland cells directly (2). This evidence is further supported by such results as are illustrated in figure 1. The amount of adrenalin injected in that instance was such as to produce a fall in blood pressure (3)—an effect due to relaxation rather than to contraction of the arterioles. We are justified, therefore, on the basis of this consideration in connection with considerations previously adduced, in drawing the conclusion that the thyroid action current evoked by small doses of adrenin is an indication of secretory activity.

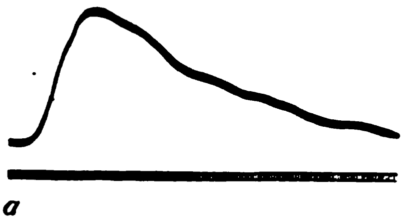

Fig. 1. Thyroid action current resulting from the intravenous injection of adrenalin (0.1 cc., 1: 100,000) at *a*. Time recorded on base line here and in other figures in half-minutes.

This conclusion accords with recent observations by Osokin (4) that adrenin injections produce marked histological changes in the cells of the thyroid—changes which he regards as of secretory rather than of pathological character. It is not in agreement with Wiener's inference (5) that adrenin leads to a storage of thyroglobulin in the gland. But this inference was based on only two cases, and the figures from these cases are so different as to make hazardous any conclusion whatever. The evidences from increase of protein metabolism after adrenin injection, from the increased histological changes of thyroid cells after such injection, and from the concordance of the action currents produced by sympathetic stimulation and by adrenin, all point clearly to a direct secretory influence of adrenin on the thyroid.

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[1] During the course of this investigation some observations by Mr. D. L. Rapport and one of us (W. B. C.) led to the discovery that stronger solutions of adrenalin (e.g., 1: 10,000) would affect other than gland tissue and smooth muscle. Thus if the galvanometer is connected with the muscles of the thigh, an intravenous injection of adrenalin (0.5 cc., 1: 10.000) will cause the galvanometer to move out continuously in the direction which would be determined by increase of the compensating current (cf. Cannon and Cattell: This Journal, 1916, xli, pp. 44, 45. This effect is not produced, however, by injections of small amounts (0.1 to 0.5 cc.) of adrenalin in dilutions of 1 to 100,000—amounts, however, which excite sympathetic endings.

The question now arises, can the secretion of the adrenal glands call forth an action current from the thyroid? The well proved power of splanchnic impulses to stimulate secretion from the adrenals makes simple an experimental testing of this question. We bared the splanchnic trunks low in the thorax, tied them together tightly at the upper end of the bared portion, crushed the nerves central to the ligature, and slipped beneath them shielded electrodes. Also a bit of twine was looped around the inferior vena cava, close to its entrance into the thorax, so that by a slight pull the blood could be prevented from returning through this vein to the heart. Now with the leading-off electrodes in place on the thyroid gland and on neighboring subcutaneous connective tissue, and the galvanometer at zero, the splanchnic fibers below the ligature were stimulated by means of a weak induced current. The record of such an experiment is shown in figure 2. The record in figure 2 *A* is that resulting when the splanchnic nerves were stimulated (at 3.34) with a weak tetanizing current for 35 seconds. The latent period was about 23 seconds from the beginning of stimulation. In figure 2 *B* (3.42) the nerves were stimulated just as they were in the case of *A*, but blood was prevented from returning from the abdomen through the inferior vena cava by a pull on the loop about the vessel. This tension was maintained for a full minute after the start of stimulation. The thyroid action current instead of appearing about 23 seconds after the stimulus was applied to the nerves (as in *A*),

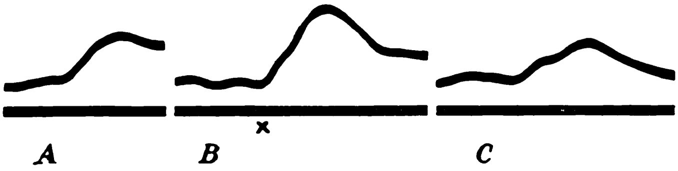

Fig. 2. *A*, 3.34. Thyroid action current resulting from splanchnic stimulation, 35 seconds. *B*, 3.42. The same, except that return of blood through the inferior cava was prevented from the beginning of stimulation (30 seconds) until a minute later (at *x*). *C*, 3.55. Thyroid action current on releasing blood from abdominal vena cava after restraining it one minute.

did not appear until the loop about the inferior cava was released (at x)—more than a minute after excitation was commenced. In all probability the higher rise in *B* (to +45) compared with that in *A* (to +30) is due to the action of some substance accumulated during the damming back of the blood in the abdomen. That retention of blood in the veins of the abdomen does not by itself produce the effects seen after splanchnic stimulation is shown by figure 2 *C*. In this instance the loop about the inferior cava was pulled upon for a minute (3.55), and then the accumulated blood was released. During the next minute the galvanometer moved slowly out to +22, and then slowly back to zero. The rise is only half as high as it was in *B*, when the same procedure was followed, but without stimulation of the splanchnics. Furthermore the maximum effects were reached nearly thirty seconds later in *C* than in *B*.

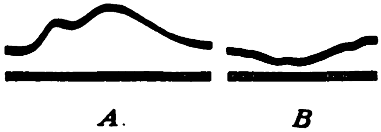

Fig. 3. *A*, 5.16. Thyroid action current following splanchnic stimulation, 30 seconds. *B*, 5.33. Absence of electrical effect on stimulating (for 60 seconds) the splanchnics after removal of the adrenal glands.

Splanchnic impulses may affect liver and pancreas as well as the adrenal glands. Discrimination of possible influences from these various sources is desirable. This can be done by noting the effect of splanchnic stimulation before and after removal of the adrenals. In figure 3 *A* is shown the record of a thyroid action current resulting from the stimulation of the splanchnic nerves (at 5.16). The galvanometer moved to +18 and to +29 and returned to +5. The adrenal glands were then carefully removed and thereupon the splanchnics were stimulated again (at 5.33) for one minute. The only effect was a wandering of the galvanometer from 0 to −4, back to 0, and to +4, as shown in figure 3 *B*. Subsequent injection of adrenalin in this instance induced a characteristic electrical change.

From the evidence illustrated above we are justified in concluding that the amount of adrenal secretion liberated by splanchnic stimulation is sufficient to excite the thyroid gland in a manner similar to its excitation by sympathetic impulses and by adrenin injections. Obviously the efficiency of sympathetic impulses in provoking activity of the thyroid might be greatly augmented by the simultaneous secretion of the adrenal glands. It is significant, therefore, that both the thyroids and the adrenals would be simultaneously stimulated by the diffusely distributed impulses of sympathetic neurons.

In an earlier paper (6) attention has been called to the emergency function of the adrenal glands in times of emotional stress, as distinguished from a routine function. The thyroid, likewise, doubtless has a routine function—one that serves to keep metabolism normal. That function can probably not be investigated by electrical methods. Because the gland is subject to sympathetic impulses, however, and because it responds to stimulation with great promptness, probably it also has an emergency function—one that is exercised particularly in times of emotional crisis. It may be that such an emergency function is an exaggerated form of the routine activity of the glands, but the determination of these suggestions must be left to future researches.

SUMMARY

Injection of small doses of adrenalin (0.1 to 0.2 cc., 1:100,000) evokes in the cat an action current of the thyroid gland.

Stimulation of the splanchnic nerves physiologically separated from the central nervous system likewise evokes an action current of the thyroid gland. This electrical change does not occur if the return of blood from the abdomen is prevented, but takes place promptly when the pent blood is released. The change also fails to appear after splanchnic stimulation if the adrenal glands have been previously removed.

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PANCREAS DEFICIENCY AND VASOMOTOR IRRITABILITY

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During the past three years the relation of various endocrine glands to the sympathetic nervous system has been under investigation in this Laboratory. That a relationship of this nature exists has been widely assumed. In view of the fact that the hormones of the body and the autonomic nervous system in general influence the same sort of functions—the so-called vegetative processes—the assumption is not at all improbable. On the other hand clean cut evidence of specific relationships between the endocrine system and either the sympathetics or parasympathetics is comparatively meagre. Particularly is this true in regard to the pancreas.

Nevertheless an important influence of the pancreas upon the sympathetic system has been asserted by the proponents of the "Wechselwirkung" theory of endocrine physiology. This assumption, in fact, forms one of the basic principles. Although the theory has largely collapsed of its own weight its occasional reappearance in supposedly authoritative clinical literature warrants some further attention to it (1).

The evidence most frequently cited of a relationship between the pancreas and the sympathetic system was recorded by Loewi in 1907 (2). After pancreatectomy both in cats and dogs it was observed that adrenin dropped into the conjunctival sac caused a dilatation of the pupil—a phenomenon that does not occur in the normal animal. The conclusion was drawn that the removal of the pancreas had caused augmented irritability of the sympathetic system, hence the pancreas normally has the function of depressing the sympathetics. Loewi further observed a similar mydriatic effect in 10 of 18 cases when adrenin was applied to the eyes of patients suffering from diabetes mellitus. In subsequent clinical literature this phenomenon is frequently referred to as the "Loewi reaction." The observation was soon confirmed by Biedl and Offer (3). Falta (4) observed adrenin mydriasis in 15 of

38 cases of diabetes. Other observers, however, have had less success. Cords (5) in 11 cases found only 3 positive. Bittorf (6) was able to obtain the reaction only twice in 10 cases and in each of these the patient was moribund. Pratt (7) found the reaction negative in three dogs with extreme atrophy of the pancreas and in a case of human diabetes. So far as we are aware observations of this sort constitute the only evidence in favor of Loewi's theory. The inconsistency of the phenomenon detracts from its significance, even granting the validity of the reasoning by which the theory was deduced.

That the deduction is valid, however, is open to question. The only condition in the sympathetic system that is known to cause an augmented adrenin reaction is peripheral *degeneration*. Elliott (8), Lichtwitz and Hirsch (9) and Biedl (10) have observed that when the sympathetic fibers to an organ are destroyed the responses to adrenin in that organ are materially increased. In view of this fact Loewi's observation as evidence is equivocal. One might equally well conclude that adrenin mydriasis, following pancreatectomy is due to depression of the sympathetic system or to a peripheral atrophy of sympathetic fibers. Indeed such an explanation has been offered to account for the glycosuria following pancreatectomy (11). On the whole it would seem to be an open question whether the pancreas has any specific relationship to the sympathetic nervous system.

In the hope of obtaining more satisfactory evidence on the point the experiments herein described were undertaken. As a typical component of the sympathetic the vasomotor system was selected as best adapted to quantitative study.

Technique. In all the experiments dogs were used as subjects. Ether anesthesia was employed throughout. By the technique described in previous papers of the series (12) the blood pressure and the reactions to standard doses of adrenin and nicotine were determined. These reactions afforded an index of the irritability of the peripheral and central and ganglionic portions of the vasomotor apparatus respectively (13). The drugs were administered intravenously. After the desired records were obtained the vessels were ligated and the incisions closed.

Then the pancreas was exposed through an incision, either in the median line or about 3 cm. to the right of this. The technique of pancreatectomy in the dog has often been described and needs no further discussion (14). The only points that seem worthy of emphasis are two: Minor hemorrhages should be ignored or stopped with hot compresses; much time can be lost in needlessly ligating smaller vessels. Also the pancreatic ducts should be identified and ligated to prevent a possible escape of intestinal contents. The glands were removed completely except for an occasional small tag of tissue. A profuse glycosuria as well as emaciation in several of the dogs that were tested showed that a severe grade of pancreatic deficiency had been produced. The incisions were closed in three layers and protected with abdominal bandages. Comparatively little trouble with infection was experienced.

At different intervals from 3 to 22 days the blood pressure and adrenin and nicotine reactions were again recorded. In most instances two or three determinations after the initial one were made on each animal.

Results. The outcome of the experiments as a whole was somewhat difficult to interpret. The most striking feature was an unusual variability in the reactions to nicotine. In this type of investigation two precautions are essential to insure constancy of vasomotor response: the anesthesia must be held at a fairly uniform depth and the drugs must be injected at the same rate each time. But with the most careful attention to these points rather marked variability could not be avoided. The general trend of the series, however, is exemplified in the following protocol:

March 23, 1916. Dog anesthetized with ether. Cannulas inserted in right femoral artery and vein. Blood pressure and reactions to adrenin 1 cc., 2 cc. (1: 100,000) and nicotine 2 cc., 3 cc., 4 cc. (1: 4000) determined. Pancreas removed. Operation and recovery from anesthesia uneventful.

March 28. Cannulas inserted in left femoral artery and vein. Blood pressure and reactions to adrenin and nicotine taken as before.

March 29. Same procedures as March 28.

Results. (*Pressor reactions to drugs*)

BLOOD PRESSURE	BEFORE OPERATION	FIVE DAYS AFTER OPERATION	SIX DAYS AFTER OPERATION
	110	120	110
	mm. Hg.	*mm. Hg.*	*mm. Hg.*
Adrenin 1 cc.	32	38	36
Adrenin 2 cc.	42	62	50
Nicotin 2 cc.	8	23	10
Nicotin 3 cc.	30	33	22
Nicotin 4 cc.	62	34	26

In most cases the adrenin reaction was augmented—sometimes 50 per cent but occasionally it was unaffected or even slightly reduced. This variability of result corresponds with that reported when adrenin is applied to the conjunctiva except that augmentation of the pressor reaction is somewhat more frequent.

As to the nicotine reaction it is difficult to come to any conclusion as to magnitude on account of the variability encountered. When the proper precautions are taken a normal dog will give essentially the same response to a given small dose of nicotine several times in succession. After pancreas extirpation, however, no such constancy obtains. For instance note the reactions to two successive doses of nicotine shown in the accompanying figure. Our first interpretation of the phenomenon was that in the weakened condition of the animal its sympathetic motor neurons were especially susceptible to deleterious influences and were quickly depressed by the nicotine. With further experience, however, it was learned that the variability might go in either direction; sometimes the first and sometimes the second reaction was greater. As nearly as could be judged under these circumstances

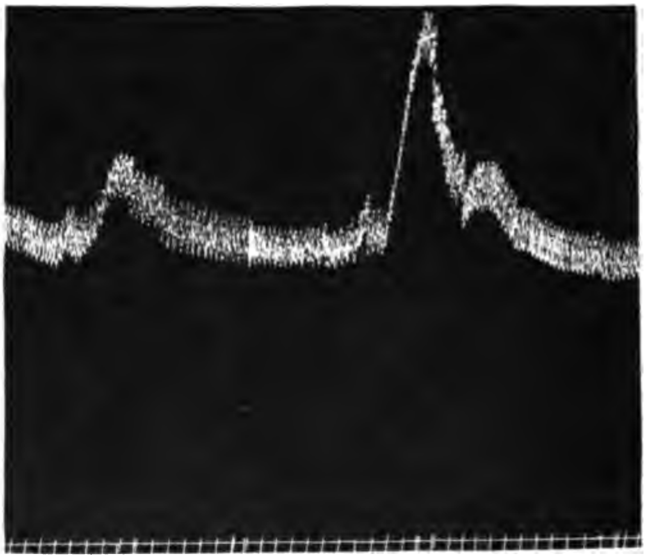

Fig. 1. Two successive vasomotor reactions to intravenous injections of nicotine 3 cc., 1: 4000. Interval, 5 minutes.

the effect of the operation was on the whole to depress the nicotine reaction.

The blood pressure was either practically unaffected by the pancreatectomy or somewhat reduced. In only one instance was any augmentation noted. The pressure seemed to depend upon the general vitality of the animal rather than on any changes in irritability of the vasomotor apparatus.

Discussion. The outcome of the experiments is not without significance in its bearing on Loewi's theory. So far as pupillary diameter is concerned one has no way of judging what it would have been had the pancreas not been extirpated. The augmented reaction might conceivably in itself amount to over activity of the sympathetic system as Loewi and others have supposed (15). This might be true even though the sympathetic neurons were functioning merely to a normal extent because a given stimulus acting through a hyperexcitable effector would cause an augmented functioning of the given organ. As a matter of fact the proponents of the theory assumed that sympathetic functioning is normally due to stimulation by circulating adrenin—a theory no longer tenable (16). This is not, however, an assumption necessary to the theory if it be a fact that normal sympathetic impulses are transmitted through the medium of the same neurocellular receptive substance as that involved in reactions to adrenin. We are aware, however, of little specific evidence on this point. But in any case, our results indicate that the theory under discussion is untenable. There is no correlation between actual blood pressure and the augmented reaction to adrenin following pancreatectomy. The pressure is either unaffected or reduced. In no instance did we note any evidence of a true "sympathticotonie."

In connection with the observed instability of the reaction—due supposedly to variations in the irritability of the sympathetic motor neurons we are reminded of certain observations made by Carlson (17). This investigator found that waves of augmented irritability sweep over the nervous system during gastric hunger contractions. Luckhardt's (18) observation that hunger contractions after pancreatectomy are greatly augmented would seem to add some weight to the suggestion that both Carlson and we have encountered the same phenomenon. One of the most marked instances of variability in reaction was observed, however, in a dog which had considerable food in its stomach—when hunger was supposedly in abeyance.

Summary and conclusion. After pancreatectomy in the dog the blood pressure remains either normal or somewhat depressed. The reactions to standard injections of adrenin are usually augmented. The reactions to nicotine become markedly variable, at times augmented, but in general depressed. The observations do not support the theory that the pancreas normally exerts a depressing influence upon the sympathetic nervous system.

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VASOMOTOR REACTIONS FROM STIMULATION OF THE FLOOR OF THE FOURTH VENTRICLE

Studies in Vasomotor Reflex Arcs. III

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So far as we have been able to learn no serious attempt has been made to locate the vasomotor center since the work of Dittmar in 1873 (1). It is known that when the brain stem is gradually sliced away from above downward no effect on blood pressure is produced until the middle of the pons is reached, when the blood pressure begins to fall. When the sections pass through the upper part of the medulla the blood pressure falls to its lowest level, a level which is not affected by subsequent section. This experiment according to the generally accepted teaching, indicates the location of a vasoconstrictor center, which is responsible for the maintenance of blood pressure, in the lower part of the pons.

In recent years our knowledge of the vasomotor reflexes has been greatly extended by numerous researches which have dealt with the changes produced in the caliber of the blood vessels by the stimulation of various sensory nerves. In spite of all this work on the vasomotor reflexes little has been done to clear up our conception of the reflex arcs involved.

In connection with the more recent detailed studies of the vasomotor reflexes evidence has been presented to show that there is probably a vasodilator center more or less separate from the vasoconstrictor center (2). Porter (3) goes so far as to say that there is a vasotonic center which is distinct from the vasoconstrictor center. It would seem that there may be as many as three vasomotor centers and the question of their location and interrelation is the fundamental problem in this field of physiological research.

We wish to record very briefly by way of a preliminary communication, the results which we have obtained by direct stimulation of the floor of the fourth ventricle. (4) These results suggest many interesting problems which must be investigated before they can be properly interpreted, yet the facts in themselves seem sufficiently interesting to merit a short report.

In six cats after ligation of the carotids the skull over the cerebellum was opened and the cerebellum removed, exposing the entire floor of the fourth ventricle from the aqueductus cerebi to the obex. The cats lost relatively little blood and showed no signs of shock, the blood pressure at the end of the operation being practically normal, varying from 100 to 160. Hemostasis was perfect and the floor of the ventricle was easily kept dry and clean. The stimulus was applied through a needle electrode. The second wire was attached to a piece of gauze moistened in normal salt solution and sewed under the skin of the cheek. The source of the current was a Stoelting inductorium No. 7090 through the primary of which a half ampere current was passed. This instrument has been roughly standardized by previous physiological experiments. With a half ampere current passing through the primary and with the secondary coil at 15 it delivers through standard platinum electrodes a stimulus just sufficient to produce a typical depressor reflex from the sciatic nerve. The coil must be moved up to 5 to produce from the sciatics the maximum pressor reflex. In these experiments we worked with the secondary coil at positions varying from 15 to 10, that is to say with weak stimuli which if applied to the sciatic through standard platinum electrodes would produce a drop in blood pressure.

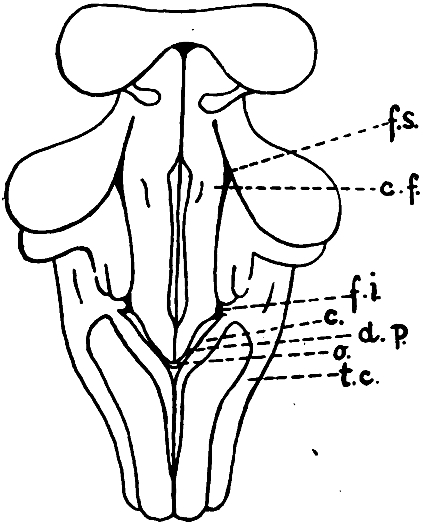

Fig. 1. Diagram of the floor of the fourth ventricle of the cat. *f.s.*, fovea superior; *c.f.*, colliculus facialis; *f.i.*, fovea inferior; *c.*, clava; *d.p.*, depressor point; *o.*, obex; *t.c.*, tuberculum cinereum.

On going over the floor of the fourth ventricle point by point with the needle electrode we found that there were two points which responded to this stimulation by producing marked changes in blood pressure. The first of these is the apex of the ala cinera or the fovea inferior, figure 1. When the electrode is applied to this point a marked rise in blood pressure occurs, often as much as 40 mm. Hg., figure 2. This response may or may not be accompanied by respiratory inhibition, excessive respiration, cardioinhibition or slight movements of the head according to the stage of anaesthesia and other variable factors. The rise of blood pressure is characteristic and specific. This pressor point, as we shall call it until we know more about its real nature, does not seem to be more than a millimeter in diameter but is readily located because the ala cinerea and fovea inferior are both readily distinguished.

The second spot, which we shall call the depressor point, is located in the area postrema just lateral to the obex. The needle is inserted under the clava just lateral to the obex, figure 1 d.p. There is no sharply defined anatomical demarcation of this as was the case with the pressor point and some experience is required before one can locate it with regularity and ease. It also seems to be relatively small and sharply localized. Stimulation here causes a marked drop in blood pressure often amounting to 35 mm. Hg., figure 2.

We have sometimes obtained a rise in blood pressure from stimulation in the region of the facial colliculus, i.e., in the neighborhood of Ditmar's vasoconstrictor center, and we have also obtained a good pressor curve from stimulation of the tractus spinalis of the trigeminal nerve, i.e., the tuberculum cinereum on the dorsolateral aspect of the medulla.

The most characteristic and consistent reactions are obtained from the pressor and depressor points. These are located not more than 3 mm. apart and the same strength of stimulus, which applied to the one sends the blood pressure up 30 to 40 mm., when applied to the other sends the blood pressure down to 25 to 30 mm. These reactions closely simulate true pressor and depressor reactions.

Aside from these two points and the region of the facial colliculus, which gives somewhat inconstant pressor reactions, we could find no part of the floor of the fourth ventricle which would give vasomotor reactions with the weak stimuli used throughout these experiments. Stronger stimuli were not used as it was felt that results obtained with them would not be reliable.

The same results were obtained with the vagi cut as with them intact, and under curare and ether as with ether alone, except that curare tends to decrease the drop obtained from the depressor point. In one cat under curare, however, a marked drop was obtained. It will be

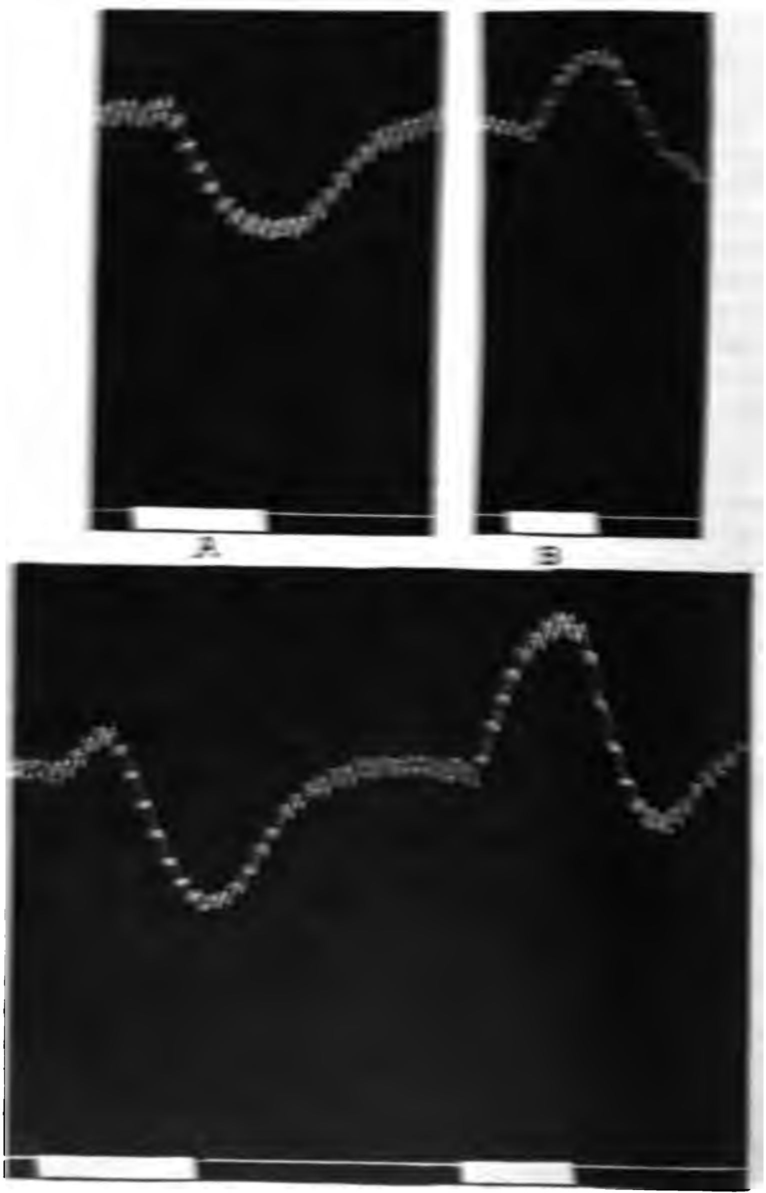

Fig. 1. Blood pressure tracing from a cat with both vagi cut and under both ether and curare. [illegible] stimulation of the depressor nerve, secondary coil at [illegible] stimulation [illegible] pressor nerve, secondary coil [illegible] stimulation of the depressor nerve [illegible] escape of current to pressor [illegible] stimulation of pressor nerve, secondary coil at 16.

remembered that curare has the effect of diminishing or obliterating the depressor reactions obtained from stimulating afferent nerves except in the case of the depressor nerve. The effect of the drug on the depressor point is therefore in line with what is known of its action in general.

All six cats gave the same results: rise of blood pressure from the pressor point, and fall in blood pressure from the depressor point. The rise and fall were always of considerable magnitude.

It would seem that there are two possibilities in regard to the nature of the points which have been found to yield vasomotor reactions on stimulation. It is quite possible that they may represent the central connections or receptive nuclei of afferent vasomotor fibers. In that case they represent a part of the afferent limb of the corresponding reflex arc. This is almost certainly the interpretation to be placed on the reactions obtained from the tractus spinalis trigemini, which is the direct upward continuation of the tract of Lissauer, which serves as the afferent path for pressor impulses in the spinal cord (5).

It is quite possible that the same interpretation applies to the pressor and depressor points which have just been described. The pressor point may represent the termination of the afferent pressor fibers of the vagus and the depressor point the termination of the afferent depressor fibers of the vagus, since both of these points are closely related to the known nuclei of the vagus nerve. The responses which were sometimes obtained from the region of the facial colliculus may have come from a stimulation of the main sensory nucleus of the trigeminal nerve which is not far distant.

On the other hand it seems possible that the depressor point may be a true vasodilator center, the pressor point a true vasoconstrictor center. In this case it would seem probable that there was also a vasotonic center in the region of the facial colliculus which was also capable of responding to direct stimulation but in a less characteristic manner than does the pressor point.

It is clear that the electrical stimulation of the floor of the fourth ventricle offers a rich field for the investigation of vasomotor reactions. Irrespective of the interpretation, which in the light of future researches is finally placed on the pressor and depressor points, a careful analysis of the reactions obtained from stimulating these points will throw considerable light on the whole question of the vasomotor reflexes.

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THE NATURE OF THE SPLANCHNIC RISE IN BLOODPRESSURE

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In 1897 Biedl (1) observed that the stimulation of the splanchnic nerve produces a vasodilatation in the suprarenal bodies, and, although unable at that time to detect vasoconstrictor fibers, the existence of fibers of this kind was inferred from the changes which result if adrenalin is injected directly into this organ. Moreover, a quantitative determination of the adrenal secretion made it appear highly probable that its formation and discharge into the venous current, is subject to the control of the nervous system, the splanchnic nerves being the secretory and vasomotor pathways of these glands. A year later Dreyer (2) succeeded in proving the existence of the vasoconstrictor mechanism in a more convincing manner and also showed that the stimulation of the splanchnic fibers leads to a liberation of adrenal substance which is quite independent of the alterations in the vascularity of these glands.

Since the publication of these papers the existence of secretory nerves to the adrenals has been established with much greater definiteness by Ascher (3), Cannon (4), Elliott (5), and Von Anrep (6). The experiments of Ascher were made upon rabbits whose abdominal organs, with the exception of the liver and suprarenal bodies, had been removed. It was noted that the stimulation of the splanchnic nerve resulted in a rise in bloodpressure only so long as the suprarenal bloodvessels were left intact. The hypothesis that the adrenal glands share in the production of the phenomena associated with emotional states, was tested by Cannon and de la Paz by immersing strips of intestinal muscle in Ringer's solution to which was added the defibrinated blood from cats that had been kept either in enforced quietude or had previously been excited. The blood from the quiet animals always failed to relax the muscle, while the blood gathered after emotional disturbances, produced a typical relaxation. In order to prove that the cause of this difference is dependent upon a discharge of adrenin, experiments were made upon the relative relaxing power of blood from different systemic veins and that of the suprarenal veins. The former type of blood proved ineffective, moreover, the relaxation failed to appear, if the blood had been obtained from "excited" animals whose adrenal bodies had previously been removed.

It has been observed by Johansson (7) that the rise in the arterial bloodpressure accompanying the stimulation of the splanchnic nerve, does not consist of a simple rise and fall, but shows two elevations. Lehndorff (8) states that the first of these is caused by a vasoconstriction of the bloodvessels in the organs innervated by the aforesaid nerve, and that the second summit is produced partly by an acceleration and augmentation of the heartbeat, and partly by a constriction of certain peripheral vessels. Elliott who investigated this question in the cat, ascribes the second phase of the rise to a discharge of adrenal secretion into the circulation, because the excitation of the splanchnic nerve failed to produce this elevation after the extirpation of the suprarenal glands. These results have been fully confirmed by Von Anrep, and furthermore, this author was able to show that the general vasoconstriction upon which the second rise depends, also occurs in certain organs and parts after their denervation. This fact clearly establishes the function of adrenin as an aid to the direct or nervous agency of vasoconstriction, and also explains the peculiar local reaction of the bloodvessels to internal pressure first noted by Bayliss (9).

The present experiments have been undertaken for the purpose of determining in how large a measure the splanchnic bloodvessels contribute to the first and second phases of the rise in the systemic bloodpressure following the stimulation of the splanchnic nerves. The dogs used in these tests were anesthetized with ether. The abdominal cavity having been opened in the median line, the diaphragm was pierced in the region of the crura. Artificial respiration having been instituted, the thoracic sympathetic nerve of each side was isolated and placed in shielded electrodes directly above its point of division into the greater splanchnic and abdominal sympathetic nerves. In order to avoid traction upon the nerve during the succeeding manipulations, the electrodes were introduced through the lateral extent of the lowest intercostal space. The current was applied to the intact nerve in some instances, and in others, only to the distal end of the divided nerve. The stimulations were of moderate strength and duration.

The bloodpressure was recorded by a mercury manometer connected with the femoral artery. A second manometer was added at times to register the pressure in the carotid artery. The following bloodvessels were placed in loose ligatures: the right and left renal arteries and veins, the mesenteric arteries, the coeliac artery, the portal vein at the hilus of the liver, and the right and left suprarenal veins.

By measuring the bloodflow through different abdominal organs during stimulation of the thoracic sympathetic, Burton-Opitz (10) has shown that the right nerve innervates, the right kidney and suprarenal capsule, as well as the stomach, intestine, spleen and pancreas, while the left nerve governs the left kidney and suprarenal body, and in addition, the portal organs just enumerated. In accordance with this distribution of the splanchnic fibers, it was thought certain that the exclusion of all of these organs from the circulation, together with the division of the hepatic plexus, would completely nullify the rise in bloodpressure which accompanies the stimulation of these nerves. Moreover, by permitting only the suprarenal veins to remain open, we hoped to obtain a means for differentiating between the effects of the discharged adrenin upon the bloodvessels of the splanchnic organs and upon those situated in other parts of the body.

At the outset, the right and left nerves were tested as to their relative effectiveness; currents of the same strength and duration being applied to them alternately. We have obtained more characteristic and more decided rises by the excitation of the right nerve, and naturally, by far the greatest amplitude of reaction was noted when both nerves were stimulated simultaneously. In a comparison of this kind, however, it must be taken into account that the rises obtained early in the course of an experiment, are generally much larger that those gotten later on, unless the intervals are sufficiently long to permit of not only a complete relaxation of the bloodvessels, but also of a thorough restoration of the secretory power of the adrenal glands.

In accordance with the preceding statements it cannot surprise that the stimulation of the splanchnic nerves during the occlusion of the suprarenal veins failed in all cases to elicit the secondary rise in bloodpressure. Quite similarly, it is possible to delay the appearance of the second phase by temporarily blocking the suprarenal vein of the gland stimulated. As is clearly indicated in figure 1, an obstruction for only a few seconds made directly at the beginning of the stimulation, suffices to retard the development of the second elevation to such an extent, that it occurs much later in the course of the vascular relaxation. In this way the first and second phases may be separated more widely so that a distinct notch or step appears between them. The general outline of the curve may thus be changed almost at will.

Strehl and Weiss (11) have stated that the ligation of the suprarenal bloodvessels is followed by an immediate fall of pressure, while the release of the ligature is succeeded by a rise. This result would tend to show that the adrenal secretion is poured out constantly to serve as a stimulant to the sympathetic nervous system and to the smooth musculature of the bloodvessels. Young and Lehman (12), on the other hand, have observed but a very slight fall in pressure, while Hoskins and McClure (13) report that the ligation of the adrenal glands causes no immediate fall in bloodpressure. The experiments of these authors, however, were not continued for a longer period than ten or thirty minutes and a subsequent removal of the ligatures was regarded as useless, because it was thought likely that the checking of the adrenal circulation results either in a depression of the function of the glands, or in an accumulation of elaborated secretion. These tests, there-

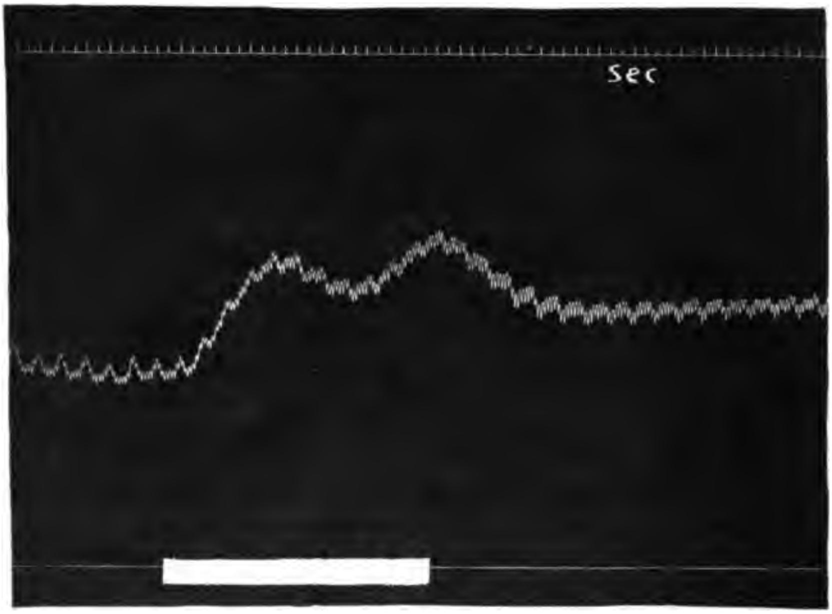

Fig. 1. Stimulation of both thoracic sympathetic nerves. Separation of the phases by brief occlusion of the suprarenal veins.

fore, do not favor the view that the constrictor and other sympathetic fibers are constantly kept under the tonic influence of the adrenal bodies.

In the course of these experiments we have had occasion to test the effect of obstructing the suprarenal bloodflow a number of times. In each case we have done so by tightening the loose ligatures which had previously been placed around the vein centrally as well as distally to the gland. Under ordinary conditions, however, it suffices to ligate only the central portion of the vein, because it seems that an escape of adrenin through distal anastomosing bloodvessels cannot readily be effected. At least, if a reversal of the venous flow of this region follows the blocking of the suprarenal vein near its outlet, the adrenal secretion reaches central channels in a wholly ineffective condition, as is evidenced by the absence of the second phase of the rise in the general bloodpressure. In testing this point we have employed solely currents of very moderate strength and duration, and hence, these results should not be applied to those cases in which the accumulated quantity of adrenal secretion has been greatly augmented by repeated stimulations of the splanchnic nerve.

While we cannot substantiate the observation that the ligation of the suprarenal bloodvessels is followed by an immediate and marked fall in pressure, the largest number of our records unmistakably indicate a downward tendency on the part of the pressure. The most pronounced drop that we have obtained is represented in the curve illustrated by figure 2. It can readily be seen, however, that its value is inconsiderable and that it develops with extreme slowness. For this reason it is easily modified and destroyed by changes in any one of the factors upon which bloodpressure is dependent; and hence, it is usually compensated for within a comparatively short time. Thus, it may happen that the initial decline is soon followed by a pressure which even surpasses that registered before the ligation.

The fact that the subsequent release of the adrenal secretion produces a rise in pressure is considered of little significance by Hoskins and McClure, because it is regarded as probable that the checking of the secretion results either in a depression of the glands, or in an accumulation of elaborated adrenin. In either case, it is stated, the removal of the ligatures does not restore normal conditions. While these possibilities must be taken into account, it seems, however, that indirect evidence of this kind should not be disregarded altogether. The same objection might indeed be raised against very many physiological methods and results.

It has been our experience that, if the ligation of the suprarenal veins was continued for only a brief period of time without stimulation of the splanchnic nerves, the release of the ligatures was not followed by a marked elevation of the bloodpressure. A certain gradual upward tendency of the pressure was, however, clearly recognizable, but again, the rise as a whole did not assume significant proportions. If the obstructions were continued for fifteen to thirty minutes, much more immediate effects were obtained which consisted in brief but quite distinct elevations of the pressure, but naturally, the question whether the adrenin has accumulated in these cases in consequence

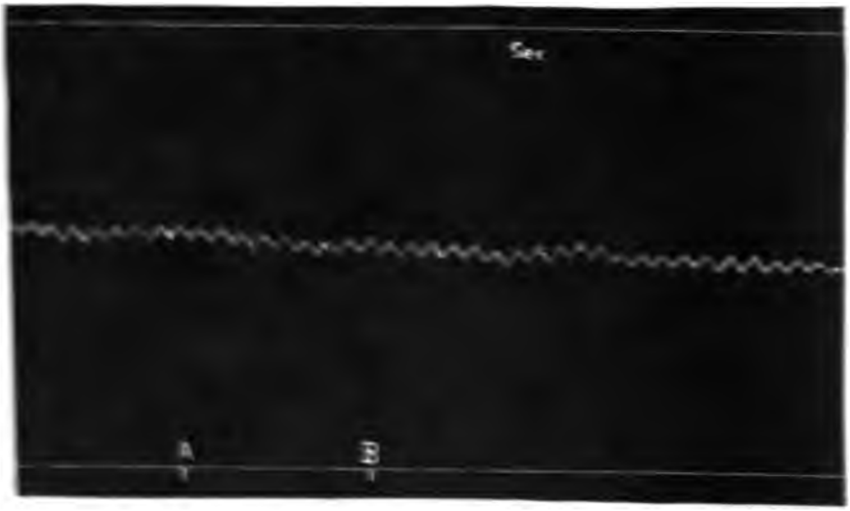

Fig. 2. Ligation of right (A) and left (B) suprarenal veins

of constant or occasional stimuli, does not find an answer in these data. Reactions of very much greater amplitude resulted, if stimulation of the splanchnic nerves had been resorted to during the obstruction. As is apparent in figure 3, on releasing the elaborated secretion first from one and then from the other gland (at A and B) two successive elevations in the pressure resulted, the amplitude of which preserved a direct relationship to the strength and duration of the preceding stimulations. The latent periods are well marked in both cases.

In accordance with these results it seemed possible to alter the general outline of the splanchnic rise in bloodpressure still further by

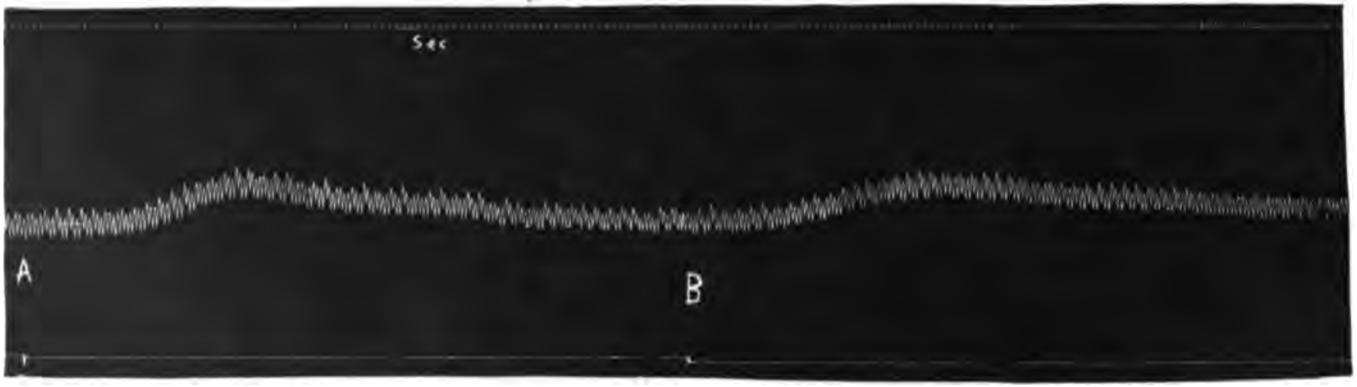

Fig. 3. Removal of the ligatures from right (*A*) and left (*B*) suprarenal veins.

excluding the effects of the primary vasoconstriction. This end we have sought to attain by temporarily ligating all of the arteries supplying the organs innervated by the splanchnic nerves, namely, the right and left renal, the superior and inferior mesenteric, and the coeliac. In addition to these bloodvessels, ligatures were also applied to the renal and portal veins, and, in a few cases, also to the hepatic vein, because it was thought possible that the resulting vasoconstriction might force a part of the residual blood of these organs into central channels; thereby tending to preserve the first rise.

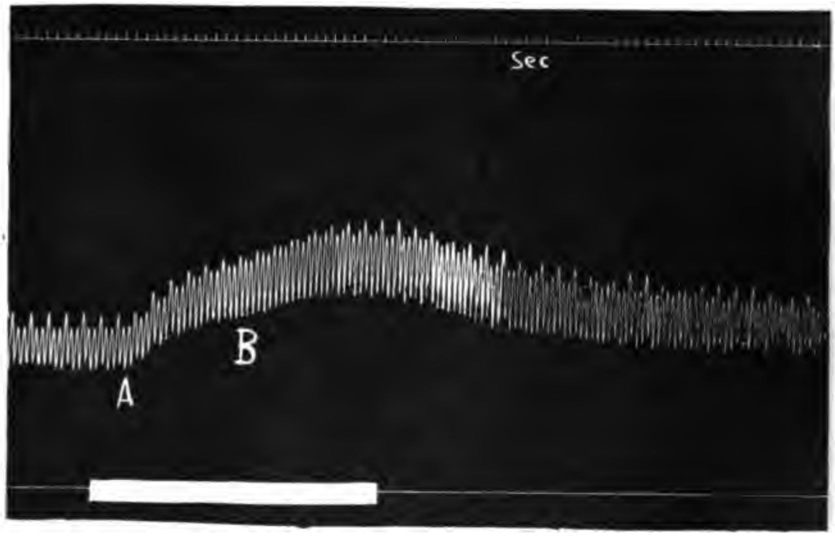

Fig. 4. Stimulation of the distal ends of the thoracic sympathetic nerves (14 cm.). All blood vessels open.

The successive exclusion of these organs from the circulation increased the general bloodpressure by about 35 to 45 mm. Hg, the pressure retaining its high value without much compensation. The greatest rise resulted after the occlusion of the mesenteric arteries. At best it amounted to about 20 mm. Hg, while that produced by the ligation of the coeliac axis, amounted to about 15 mm. Hg. By removing the ligatures one at a time quite similar step-like decreases in pressure were obtained which showed the same relationships toward one another. In all these cases, however, the portal vein must be closed last, otherwise a large part of the blood will be stagnated in the tributaries of this vessel with the result that the general bloodpressure assumes a level much below normal.

The splanchnic organs having been excluded from the circulation in the manner just described, the stimulation of only one or both thoracic sympathetic nerves produced in most of the animals a very marked reduction in the amplitude of the first elevation. In a few cases the first rise disappeared altogether. The second elevation, on the other hand, was never absent, although always smaller in size. This change, however, is explicable without difficulty upon the basis that the second summit is really founded upon the first and hence, its conspicuousness must decrease in accordance with the loss in the amplitude of the first. The accompanying figures 4 and 5 are intended to illustrate this point more fully. The former was recorded with the bloodvessels opened and the latter after they had been temporarily occluded. In each case both nerves were stimulated simultaneously with a current of medium strength and duration. The second rise which, as has been stated previously, is dependent upon the discharge of adrenin, begins about fifteen seconds after the onset of the stimulation at *B* and ends sometime after the cessation of the excitation. The first rise which is caused by the primary vasoconstriction of the bloodvessels of the "splanchnic" organs, becomes evident at *A* within two or three seconds after the beginning of the stimulation. Figure 6 is intended to illustrate the results which were obtained in only two of the eight animals experimented upon. No rise whatever is discernible in this case until the adrenin has had sufficient time to produce the characteristic second elevation (at *B*).

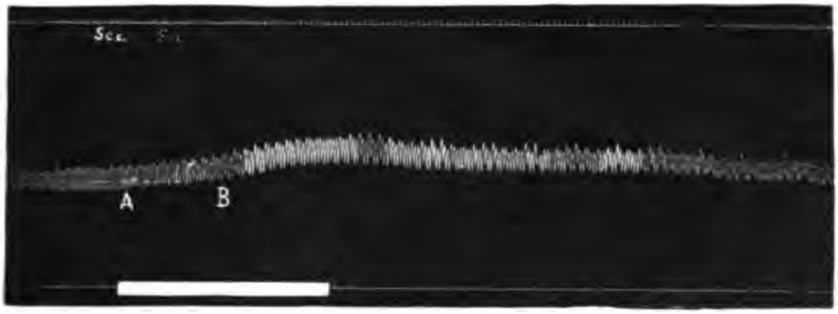

Fig. 5. Stimulation of the distal ends of the thoracic sympathetic nerves (14 cm.). Only suprarenal veins are open.

The reduction in the amplitude of the rise in bloodpressure is quite apparent in all cases, but what concerns us most at this time is the fact that the primary elevation cannot always be completely destroyed by the ligation of the splanchnic bloodvessels. In fact, other tests have given even greater rises, equalling 8 to 12 mm. Hg. Clearly, therefore, the most important part in the production of the first rise is played by the bloodvessels of those organs which are innervated by the greater splanchnic nerves. Obviously, however, the excitation of the thoracic sympathetic nerve must also affect another vascular area which is not situated in the domain of the splanchnics. This fact was brought out more clearly by ligating the suprarenal veins in addition to the bloodvessels mentioned previously. The stimulation of the thoracic sympathetic nerves produced under these conditions no elevation whatever in two animals, but a very evident first rise in six animals.

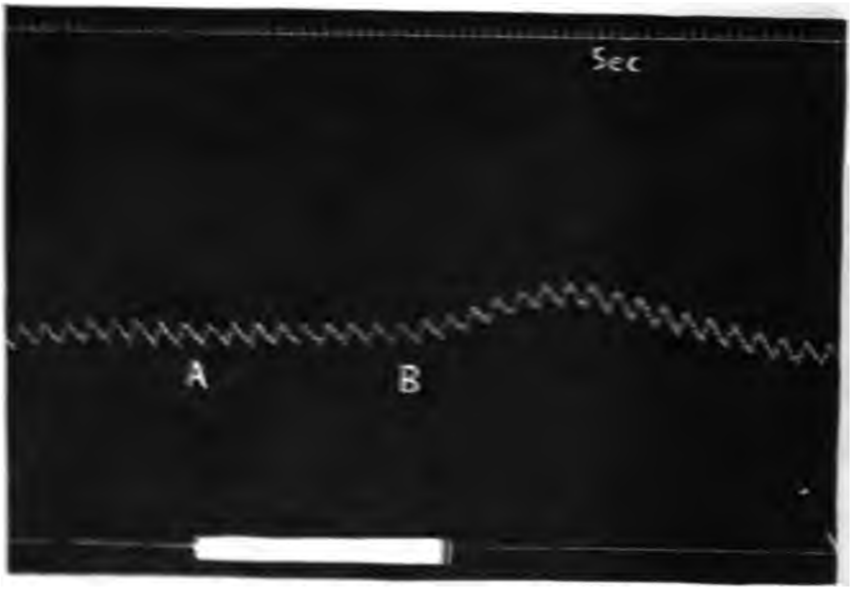

Fig. 6. Stimulation of the distal ends of the thoracic sympathetic nerves (14 cm.). Only suprarenal veins open

As is well known, the thoracic sympathetic nerve divides close to the diaphragm into the greater splanchnic and abdominal sympathetic nerves. In its course downward, the latter gives off branches which enter the ramification of nerves in the vicinity of the suprarenal capsule. It is true, however, that the size and location of these nerves is subject to variations; in fact, in some animals the abdominal sympathetic is very rudimentary.

In order to trace these extra-splanchnic vasomotors, the existence of which seems to have been definitely established by the preceding experiments, we resorted first of all to division of both greater splanchnic nerves. All the different splanchnic bloodvessels having been ligated, the stimulation of the thoracic sympathetic nerves now led to results

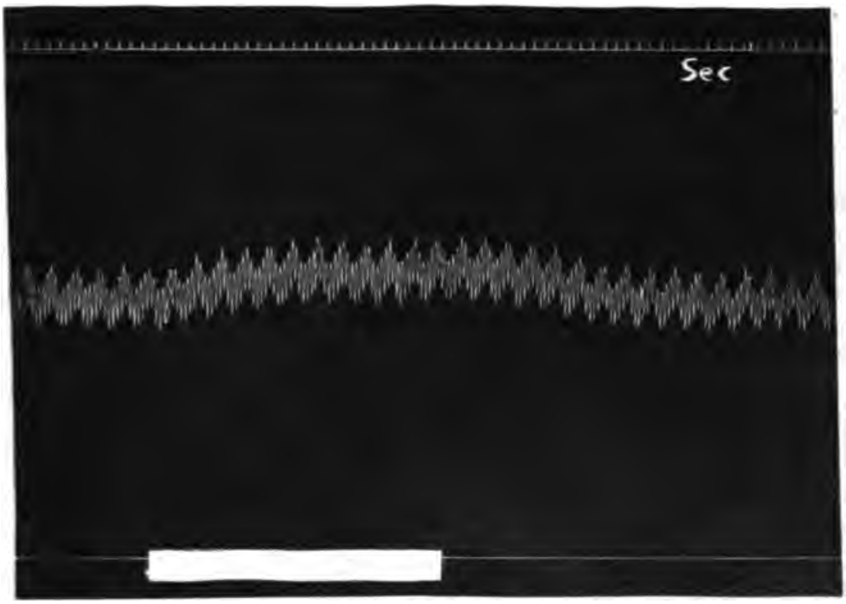

Fig. 7. Stimulation of the thoracic sympathetic nerves (12 cm.). Greater and minor splanchnic nerves are divided.

which were in no way different from the preceding. In the largest number of instances a clearly perceptible first rise developed, while in those animals in which no reaction was obtained, the abdominal nerve was poorly developed.

This procedure, however, does not exclude the possibility that impulses generated upon the thoracic sympathetic reach the splanchnic organs by way of the lesser splanchnic nerves, although the ligation of the bloodvessels would prevent an effect of this kind from making itself felt centrally. In order to avoid this difficulty, the stimulations of the thoracic sympathetic nerve were also repeated after division of the greater and lesser splanchnic nerves, and after all the bloodvessels had been ligated. As is shown in figure 7, the first phase of the rise in bloodpressure persisted in most of these tests, a low and long elevation now taking the place of the formerly very conspicuous double rise.

These results indicate that the abdominal sympathetic nerve embraces vasoconstrictor fibers which govern the vascularity of parts not situated within the realm of the greater and minor splanchnic nerves. Hence, the first elevation of the rise in the general bloodpressure resulting from stimulation of the thoracic sympathetic nerve, is in part dependent upon the constriction of the bloodvessels innervated by this nerve. It is true, however, that the power of the abdominal branch of the sympathetic is very much less than that of its splanchnic division, a difference which no doubt is dependent upon the relative size of the vascular areas controlled by these nerves. While we have not been able so far to determine the seat of this reaction, we have thought it probable that the abdominal sympathetic may be functionally connected with the lumbar and sacral networks of the sympathetic system and may thus serve as a constrictor of the bloodvessels of the lumbar muscles.

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DEPRESSOR ACTION OF THE THORACIC SYMPATHETIC NERVE AND ITS BRANCHES

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It has been noted repeatedly that the rise in the systemic bloodpressure occasioned by stimulation of the splanchnic nerve, is followed at times by a fall below normal which may in turn be succeeded by a slight rise. This phenomenon has generally been regarded as being dependent upon a compensatory adjustment in distant parts of the vascular system. It is true, however, that the fall attains at times a conspicuousness which actually suggests a reflex dilatation. A reaction of this kind is represented in figure 1. The current was applied in this case to the intact thoracic sympathetic nerve, directly above its point of division. The stimulation was followed at first by a rise in bloodpressure consisting of two phases (at *A* and *B*), while subsequent to the second elevation the pressure dropped rather abruptly (at *C*), giving rise to an effect which closely resembles a true depressor action.

In other parts of the body Dastre and Morat (1) have observed that the excitation of a so-called constrictor nerve produces a constriction of the bloodvessels innervated by this nerve and that this reaction is followed by a dilatation. This after-affect to which the name of "surdilatation" has been given, is considered by them to be an exhaustion phenomenon. It is assumed that the excitation increases the tonicity of the muscle fibers of the vessel wall in such a degree that they become fatigued and temporarily relax.

Bradford (2) has shown that the vasomotor nerves of the kidney leave the spinal cord through the anterior roots from the fourth to the thirteenth dorsal vertebrae. These fibers embrace constrictors and dilators, but the former are the best developed by far so that it is difficult to obtain clear evidence of vasodilation by stimulation of a spinal nerve. Excitation of the central end of an intercostal nerve was followed by a contraction of the kidney, the same result being usually obtained by reflex stimulation of the spinal nerves. As far as the splanchnic nerve is concerned, the statement is made that this nerve embraces constrictor and dilator fibers for the abdominal bloodvessels, because it was possible to induce a fall in the general pressure which was frequently succeeded by a rise. In accordance with the mode of preparation, these effects have been attributed by Bradford to a

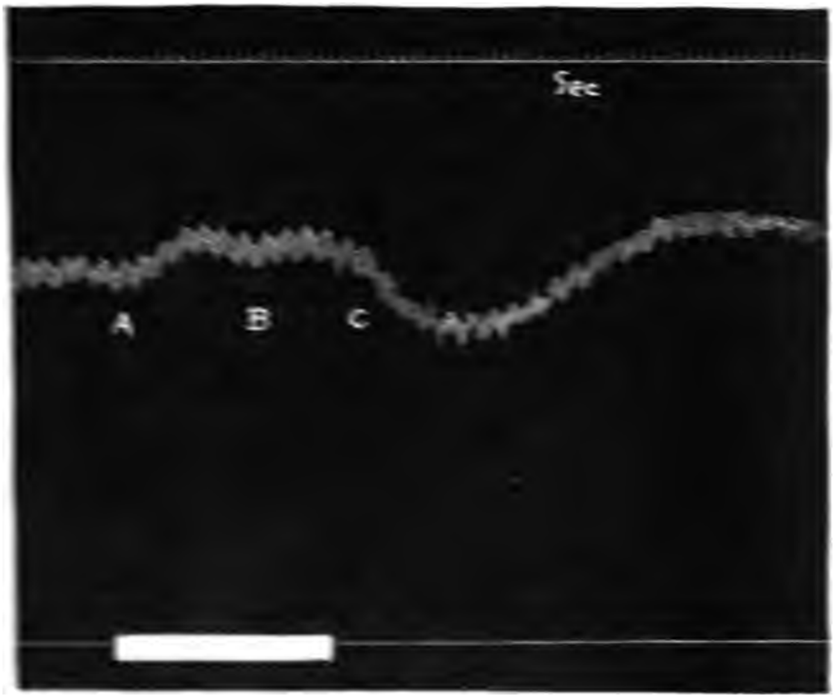

Fig. [illegible]. Stimulation of the thoracic sympathetic nerve [illegible] cm.

peripheral and not to a central excitation. The shrinking of the kidney, for example, is explained by assuming that the stimulation of the splanchnic nerve acts mainly upon the bloodvessels of the intestine, so that the constriction in the latter organ in turn produces a [illegible] in the bulk of the kidney. This reaction is said to take place even if the renal vessels take no part in the dilatation, because a very large quantity of blood is diverted into the intestinal channels. Thus it is believed that these dilator phenomena are dependent upon a direct and active dilatation of the intestinal bloodvessels which in turn affects the kidney in a passive manner, although it is not denied by Bradford that this organ may also show some active dilatation.

The phlethysmographic experiments of Hallion and Francois-Frank (3) made upon loops of intestine, have shown that the vasoconstrictor fibers of the intestinal bloodvessel arise below the fifth dorsal nerve, while the dilators take their origin in the eleventh, twelfth and thirteenth dorsal communicating rami as well as in the first and second lumbar nerves. The stimulation of the distal ends of these fibers produces an increase in the volume of the intestine which may and may not be initiated by a slight decrease. The former reaction is associated with a rise and the latter with a variable fall in the aortic pressure.

I have cited the work of these authors somewhat in detail in order to show that it is wholly concerned with direct peripheral effects, namely with the vasoconstrictions and vasodilatations occurring in the kidneys and intestines. In the succeeding pages I shall endeavor to prove that the "splanchnic" nerve also serves as an afferent path and that its excitation leads to a general vasodilatation. For this reason, this nerve may be regarded as the depressor for the abdominal organs.

Opposite to the thirteenth rib, the thoracic sympathetic divides into the greater splanchnic and abdominal sympathetic nerves. Distally to this point, the border-sympathetic most generally consists of only a small number of fibers which eventually connect with the lumbar ganglia, but at times it retains a size which compares favorably with that of the splanchnicus.

The dogs made use of in these experiments were anesthetized with ether. Having opened the abdominal cavity, the diaphragm was pierced on both sides in the regions of the crura. The bloodpressure was registered by a mercury manometer which was connected with the left femoral artery. The stimulation of the nerves was effected with a Du Bois-Raymond coil and ordinary shielded electrodes; a three-volt battery furnished the power.

To begin with, the electrodes were applied to the intact thoracic sympathetic nerve about 1 cm. above its division into the greater splanchnic and abdominal sympathetic nerves. The coils were at first widely separated so as to be beyond the threshold value of the current. They were then brought closer together after each stimulation, until eventually the first indications of a fall in bloodpressure appeared. If this end could not be attained with weak currents, the frequency of vibration of Neef's hammer was reduced gradually by increasing its contact distance. A graduated inductorium and Schlagwähler were never required in these tests to bring about the result just mentioned.

The decreases in the pressure could be rendered more conspicuous by increasing the strength of the current somewhat beyond its threshold value. When, however, a certain maximum had been reached, the fall was invariably superseded by a rise in the pressure which exhibited the characteristic double elevation. In all these tests it was necessary to separate the individual stimulations by intervals of a few minutes, otherwise the succeeding falls in pressure lost much of their original conspicuousness. This difficulty which clearly suggests that the dilators are much more susceptible to fatigue than the constrictors, can be overcome by using a somewhat stronger current for the succeeding stimulation. For example, if a marked fall in pressure has been obtained with a distance of coils of 25 cm., an increase, say, to 20 cm. will tend to preserve the amplitude of the drop without necessitating a long period of rest. Quite similarly, it was possible to produce a series of rises and falls in rather rapid succession by simply increasing or decreasing the distance of the coils.

While the amplitude of the fall in pressure could be varied by changing the strength of the current, it was rather difficult to drive the pressure beyond a certain low level. In the tests here at hand, the decreases [illegible] amounted to [illegible] mm. Hg., but rarely to more than [illegible] mm. Hg. The maximal values obtained equalled [illegible] to 40 per cent of the normal pressure. In general, therefore, it may be said that these [illegible] bear every [illegible] to the fall in pressure which is [illegible] by the excitation of the depressor nerve (fig. [illegible]). In no case, however, could a marked decrease in the frequency of the heart be made out. [illegible] seemed always to exist. A [illegible] normal beats [illegible] sixty-five beats during the period of [illegible].

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constrictor properties (4); in recent years, however, many new facts have been ascertained which tend to prove that in very minute doses this agent also manifests the power of lowering the pressure by relaxing the bloodvessels. Evidence of this kind has been obtained by Bardier and Fränkels (5), Moore and Purington (6), Meltzer (7), and Pari (8); in fact, Cannon and Lyman (9) have succeeded in producing a fall in bloodpressure in cats after exclusion of the splanchnic organs by simply stimulating the splanchnic nerve or the gland itself. Reasons are given by these authors for assuming that this dilator effect is not of central origin, is not dependent upon a blocking of the constrictor impulses and is not caused by the stimulation of supposed vasodilator sympathetic endings. Thus, adrenalin is said to possess opposite actions in accordance with the state of the smooth muscle

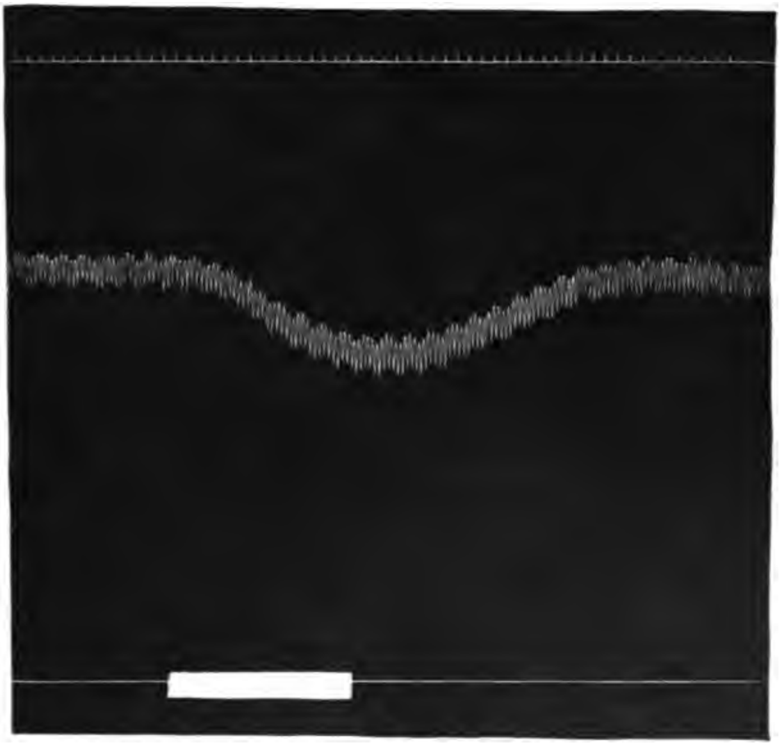

Fig. 2. Stimulation of the intact thoracic sympathetic nerve (25 cm.)

fibers of the bloodvessels. If these elements are tonically shortened this agent relaxes them, while if relaxed, it contracts them.

It has been observed previously (10) that the latent period intervening between the stimulation of the greater splanchnic nerve and the beginning of the second phase of the rise in the general bloodpressure amounts to about 13 seconds. It may be assumed, therefore, with reasonable certainty that a dilator effect which is caused by a liberation of adrenal secretion, must require an equally long time to make itself felt. An inspection of the records, however, shows that the latent periods seldom exceed a length of 3 to 4 seconds, in spite of the fact that they tend to be somewhat longer than those initiating the splanchnic rise in bloodpressure.

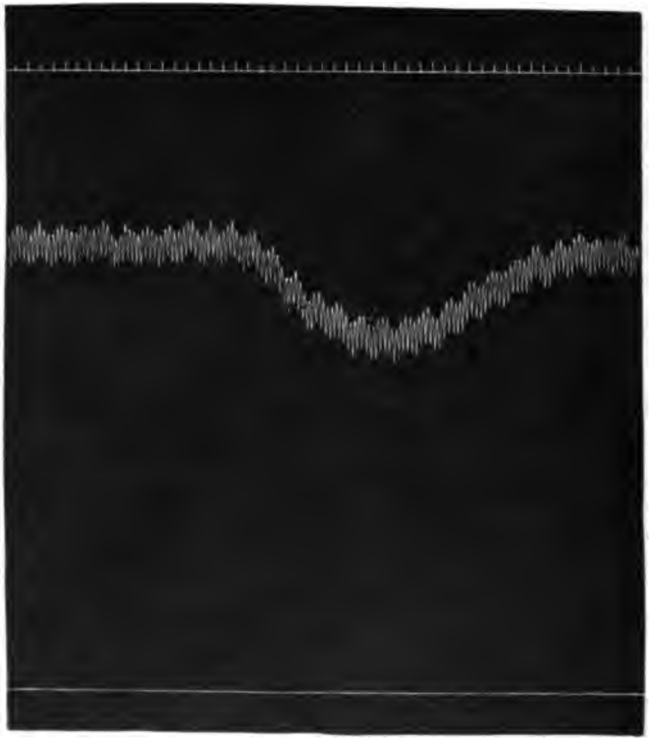

Fig. 3. Mechanical stimulation of the central end of the thoracic sympathetic nerve.

While probably no additional evidence is required to show that this depressor action is not dependent upon a liberation of minimal quantities of adrenin, I ligated in one animal the suprarenal veins centrally and distally to the glands and removed practically all of their tissue. No change whatever in the character of the fall in bloodpressure was noted.

Having in this way excluded the adrenal bodies as causative factors in the production of this vasomotor reaction, I proceeded to establish its reflex character in a more convincing manner. To begin with I divided the greater splanchnic nerve of the left side in close proximity to the suprarenal plexus and stimulated its central end in the manner described previously. Very pronounced falls in bloodpressure were obtained. I then divided the abdominal sympathetic nerve above the first minor splanchnic nerve, and repeated the stimulations, using

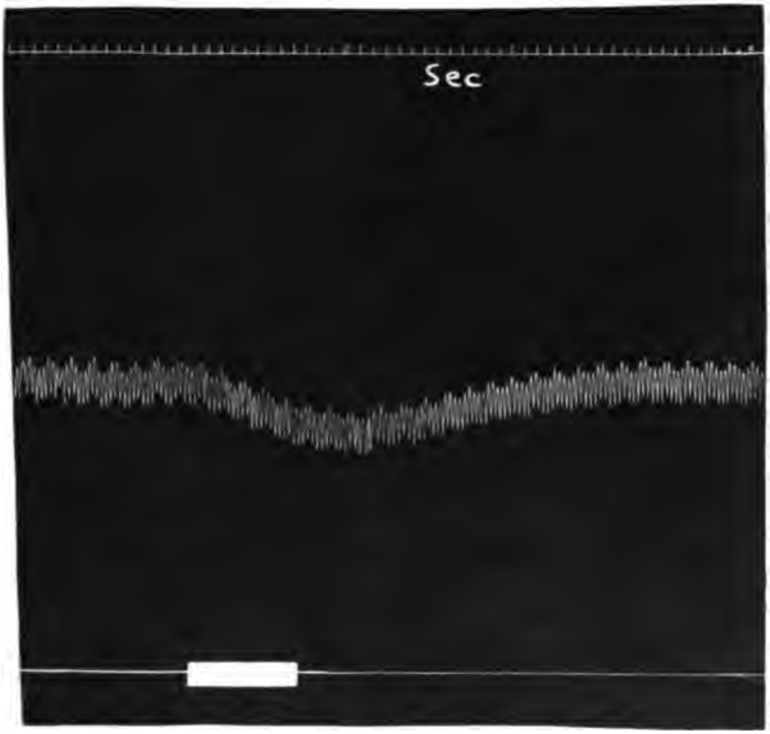

Fig. 4. Stimulation of the central end of the left thoracic sympathetic nerve, the opposite nerve and both vagi nerves having been divided (22 cm.).

the central end of this nerve. While the reactions now showed a reduction in amplitude, their general character remained the same. And finally, I divided the thoracic sympathetic nerve at a point about 1 cm. above its bifurcation and applied the electrodes to its central end. The stimulations invariably led to very characteristic falls in pressure. Precisely the same results were obtained subsequently with the right thoracic sympathetic nerve and its branches, and hence, the conclusion may justly be made that the aforesaid nerve serves as the depressor nerve for the abdominal viscera. It might also be mentioned that a mechanical stimulation of the central end of this nerve sufficed in many cases to bring this result about (fig. 3).

In accordance with the large amplitude of this depressor reaction, it was thought probable that extensive areas of the vascular system are involved, but naturally, one might feel inclined to attribute the principal effect to the bloodvessels of the abdominal organs. Without desiring to establish accurate quantitative differences, as could probably be done with the help of a calibrated inductorium (11), I have sought to determine the seat of this reaction in a perfectly general way as follows: Having divided both thoracic sympathetic nerves, the electrodes were applied to the central end of one or the other of these nerves. Both vagi nerves were then cut a short distance above the diaphragm, and also both phrenic nerves.

The stimulations invariably incited typical depressor reactions which, however, possessed a smaller amplitude than those obtained previous to the division of these nerves (fig. 4). No doubt, this change is due in some measure to the low bloodpressure occasioned by the division of the sympathetic fibers, because it was possible to again render them more conspicuous by injecting a small quantity of normal saline solution. While these results do not minimize the importance of the abdominal organs as factors in the production of this reaction, they clearly prove that the latter finds its origin in a reflex vasodilatation which involves the circulatory system in general.

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LOCALIZATION OF A PRIMARY PACEMAKER IN THE TURTLE'S HEART

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INTRODUCTION

This work was undertaken in an attempt to obtain evidence regarding the place of origin of the cardiac impulse in the turtle's heart.

HISTORICAL

The specialized tissue of the mammalian heart has been warmed and cooled by a number of workers since MacWilliam (1), and Adam (2). Until very recently such work has not been done upon the cold-blooded heart. There has been no attempt, except recently, to see whether there is a particular spot or portion in the sinus venosus of the lower animals which not only has fundamental significance in heart activity, but which would necessarily be carried forward or rather be embodied in the cardiac mechanisms of animals higher up in the scale. Certain workers have questioned the sino-auricular node as the seat of impulse formation in the mammalian heart unless it could be shown that the homologous structure of the cold-blooded forms had a similar function. The general conception has been that it did not matter what part of the sinus venosus was incorporated (developmentally in the mammalian heart) because it was thought that that in itself was sufficient, seeing that the sinus venosus (since the work of Gaskell, MacWilliam, and Engelmann), has the highest degree of automaticity of the heart. Erlanger (3) notes that in lower forms the pacemaker does not begin in the sinus node. Meek and Eyster (4) have briefly described the views on the origin of the heart-beat in cold-blooded hearts. Their own results "indicate that the point of primary negativity is in the region of the right cavo-sinal junction." They later published a preliminary communication on the origin of the cardiac impulse in the turtle's heart, "and found the junction to precede in negativity." The beat was initiated not in the entire sinus but at a definite spot, which they have further definitely located, in 1916 (5). They were the first to do for the cold-blooded heart, what Wybauw, and Lewis, Oppenheimer and Oppenheimer, did for the mammalian heart. This spot has been identified as identical histologically with the specialized tissue in the sino-auricular junction of lower forms by Keith and Flack, Keith and Mackenzie, and Laurens (see 5).

Definite localization of beat initiation of the frog's heart was undertaken by Engelmann, in 1896 (6). He concluded that the heart-beat does not arise from any definite point in sinus or veins but that any portion in them can function as pacemaker. Engelmann (7) later (1903) reaffirmed his first position.

Engelmann is the only worker who has warmed (with platinum galvano-cautery) or influenced circumscribed areas of the sinus venosus and veins in the cold-blooded heart (frogs—normal and atropinized). Gaskell was the first to heat or cool the whole sinus (turtle) and conclude therefrom that the beat originates in it.

An advance is made when cold, warm, electrical and other stimuli under definite control are applied to closely circumscribed areas of the heart, especially in the sinus venosus.

THE PROBLEM

We attempted to find a spot in the veins, sinus venosus proper, or the sino-auricular junction of the turtle's heart, which upon being properly cooled, warmed, stimulated directly by an electric stimulus, or seared, would change the rate of the heart, or stop it altogether or temporarily.

METHODS

Experiments were performed on turtles, most of them 10 and 12 inches long, all of which had been double pithed. The plastron was removed, the pericardium incised, and the frenum cut through so that the heart could be examined dorsally and ventrally. In some cases the aortae were ligatured, and cut, to free the heart entirely. In one group, the contractions of the right auricle and ventricle were recorded by air transmission through receiving tambours to recording tambours. In this group and those following, various spots on the sinus, auricles, and veins, were cooled—in some experiments with ethyl chloride, in others with pencils of ice, in some by means of a copper tip attached to an apparatus from which one could also supply the tip with warm water when needed and so warm it; and in others the spots were stimulated directly by an electric current, or warmed by the copper tip. The temperatures applied by the copper tip were carefully noted. In a second group of experiments the contractions of the sinus and right auricle were recorded directly on the drum by way of a vertical thin glass rod, with cork tip resting on the chamber, meeting a horizontal recording lever. In a third group, the various spots of the heart were seared by a red-hot rod. In a fourth group, the rate and action of the chambers were counted by direct inspection and observation, with no records being made. This was done on the heart in situ, and on the excised heart immediately after excision, or twelve to twenty hours after excision. In Experiment XX, and a few others, the ventricle was removed entirely after being ligatured at the auriculo-ventricular junction, and a forceps was placed on the auricles so that the sinus venosus itself received the entire attention of the observers. In some experiments atropine and nicotine solutions were placed on the heart directly; and in Experiment XXV, 1 per cent solutions of atropine, apocodeine, and nicotine were used.

We have felt justified in interpreting the change in rate produced by the various stimuli on the turtle's heart as an effect upon specialized tissue, because previous experiments on the mammalian heart rigidly controlled by histological examination have proven such assumption to be correct for the mammalian heart. Histological examinations of the tortoise heart show that the best connection between the sino-auricular ring and the right auricle is at the position of the right venous valve.

The first experiment was made by Mr. Schlomovitz, in the laboratory of Prof. A. S. Loevenhart, University of Wisconsin, on July 13, 1914. Five were completed there. Experiment VI and all the rest were concluded at Iowa, starting October 19, 1914, and from then on the authors were jointly interested in the work.

EXPERIMENTAL RESULTS

Figure 1 shows how the various spots were designated, and illustrates the elimination method of spots on the heart chambers until the definite one was determined which could be influenced markedly.

A. Temperature influences—heart in situ

Cooling. A definite spot was found which, upon being cooled by ethyl chloride spray, decreased the rate of the heart (fig. 2, Experiment II). This spot we have tried to localize as definitely as possible, but of course it may be possible that the cooling effect may have spread not only to the right of the median line, dorsal side, but into the adjacent sino-auricular junction. This same error may hold throughout the experiments, although at times we were able to cool a spot not more than 2 mm. in diameter with complete assurance that there was no spreading. In Experiment XVIII (at No. 18), slowing was produced by applying an ice pencil, and in Experiment XIX at (No. 37) the rate was decreased 50 per cent for a short period. Figure 3 shows the outlines of the sinus and the spots warmed, cooled, etc. In this experiment (XX), the ventricle was cut away after applying a ligature in the auriculo-ventricular junction, and a forceps placed upon the auricles, in order that the sinus movements could be accurately recorded, and certainly not interfered with by the strong ventricular pull.

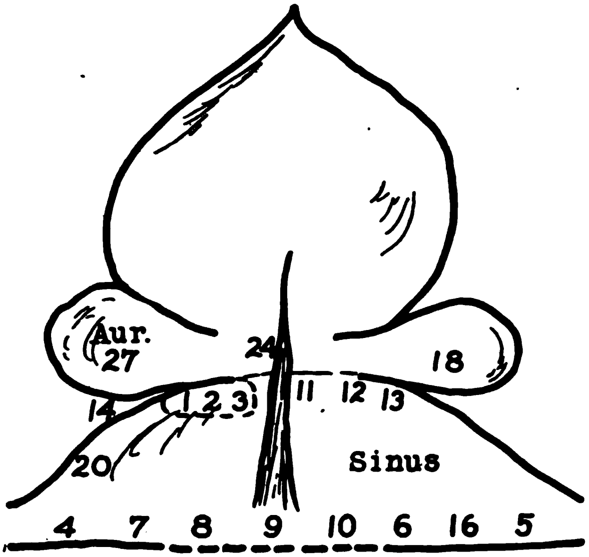

Fig. 1. Dorsal view of turtle's heart. Figure referred to in text.

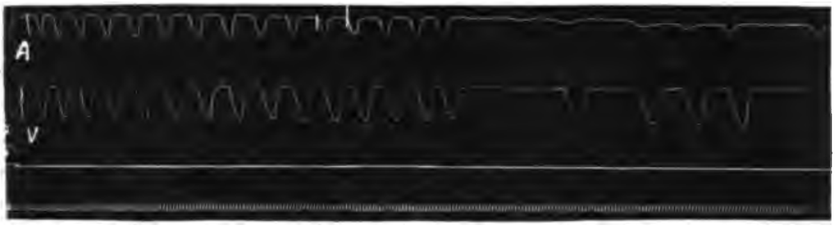

Fig. 2. Effect of localized cold on turtle's heart. Cooling with ethyl chloride. Upper record, contractions of auricle. Lower record, contractions of ventricle. Time in seconds.

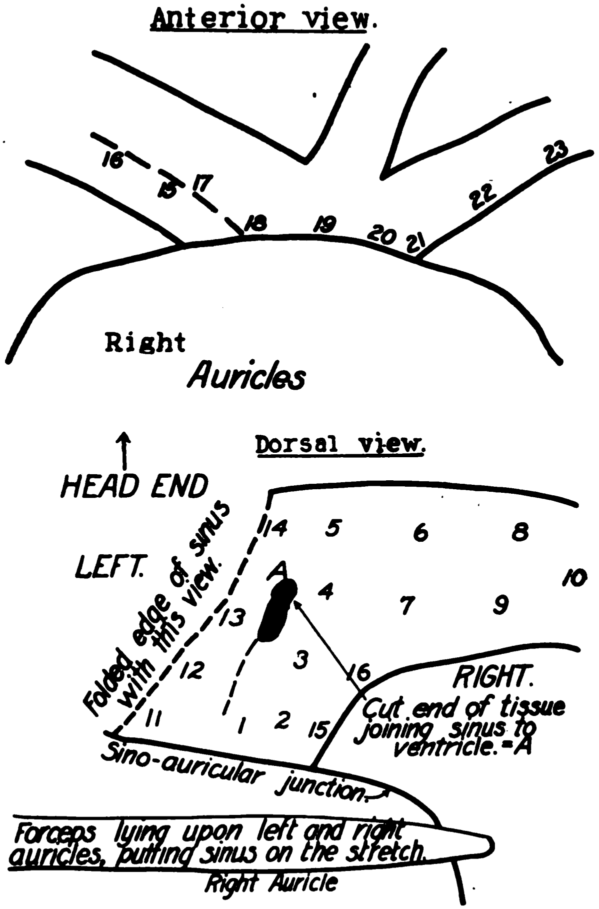
Anterior view.
16
15
17
18
19
20
21
22
23
Right
Auricles
HEAD END
Dorsal view.
LEFT.
Folded edge of sinus with this view.
14
5
6
8
A
4
7
9
10
13
3
12
16
RIGHT.
Cut end of tissue joining sinus to ventricle.-A
11
1
2
15
Sino-auricular junction.
Forceps lying upon left and right auricles, putting sinus on the stretch.
Right Auricle

Fig. 3. Sinus region of turtle's heart showing areas warmed and cooled in Experiment XX.

Warming. Warmth 35 to 40°C. produced an immediate increase in rate of the heart only when applied at the definite spot determined for that animal. This spot was in nearly each case either in the sinus venosus on the right of the median line in the S-A junction or just adjacent to it. In Experiment XX the normal rate of the sinus was 30 per minute. When spots 1, 5, 6, 7, 10, and so on, except 2, 3, and 4, were warmed, the heart rate remained 30 per minute, but at spots 2, 3, and 4, the heart rate was 48 and 54 per minute an increase of 60 and 80 per cent. In counting rates a stop-watch was used. The effective spot in this experiment is in the body of the sinus. This is irregular. When the tissue connecting the sinus and ventricle was cut at the beginning of the experiment, the auricles fibrillated for ten minutes, and then became regular again. Probably we have ourselves to blame for not exploring the S-A junction to the right of point 11 more carefully.

Table 1 is a summary of the results shown in figures 4 to 7.

TABLE 1

	EXPERIMENT	NO.	PROCEDURE	RATE PER MINUTE NORMAL	RATE DURING PROCEDURE	CHANGE IN PER CENT
Figure 4..........	XVI	84	Cooling	18	12	33
Figure 5..........	XX	53	Warmed	27	36	33
Figure 6..........	XX	36	Warmed	27	27	0
		37	Warmed	30	48	60
		38	Warmed	30	30	0
Figure 7..........	XVIII	21	Warmed	27	54	100

Excised heart. After investigating the heart's action in situ in Experiment XVIII, it was excised, and by observation no increase in the rate of auricles, ventricles, or sinus, occurred after warming a number of spots, but when a certain spot in the sinus was warmed, there was an immediate increase from 36 to 54 per minute (50 per cent). In Experiment XIX, with the heart in situ, the increase was from 30 to 54 per minute (80 per cent). The increase in rate that occurred in every case of warming with temperature 35 to 40°C., in each experiment, was rapidly, almost immediately initiated; and the increase in rate is so apparent as to almost clinch the conclusion of the observer that at that spot certainly a different kind of cardiac activity is occurring, or else a different kind of cardiac tissue is being influenced.

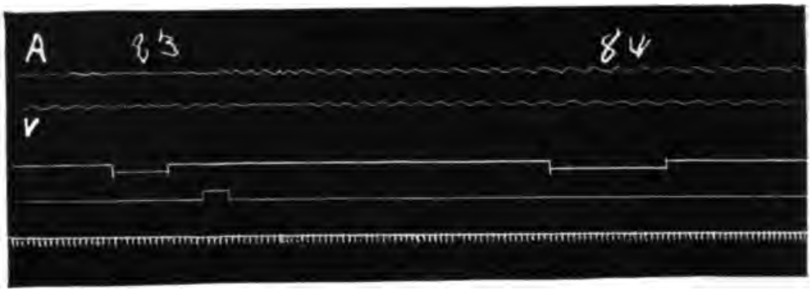

Fig. 4. First line, contractions of auricle. Second line, contractions of ventricle. Third line, signals duration of warming and cooling. Time in seconds.

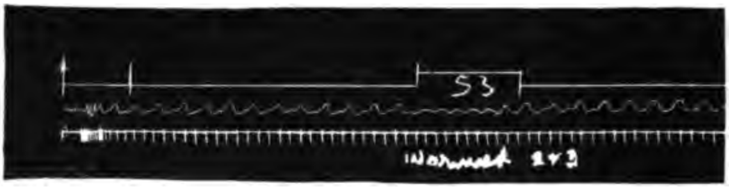

Fig. 5. Rate before 27 per minute; rate during 36 per minute.

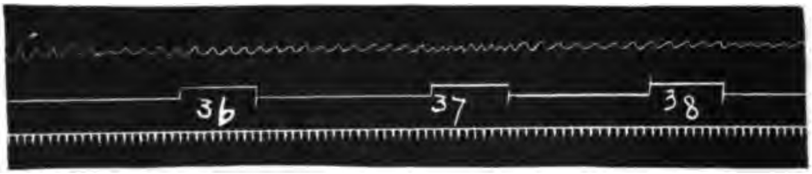

Fig. 6. Rate before No. 37, 30 per minute; rate during No. 37, 48 per minute.

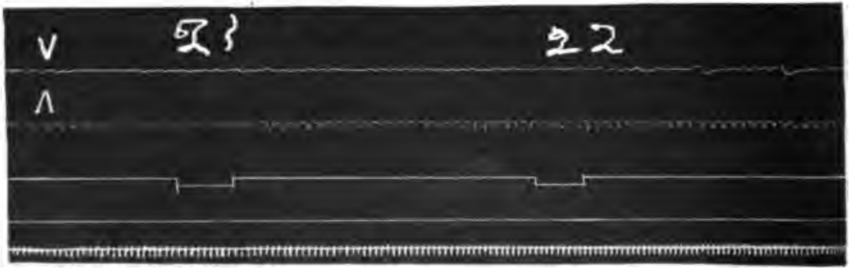

Fig. 7. Rate before—27 per minute; rate at No. 21, 54 per minute.

With an increase in temperature, 45 to 55°C., more than one spot can be found in the sinus and veins which cause an increase in rate, but there is only one spot, the sino-auricular, in which the increase is produced immediately without any latent period. In Experiment XXIV application of warmth only at the junction immediately produced fibrillation of the auricles, while at the other points including the right vein the same temperature usually produced a slight increase after a latent period.

B. *Effects of atropine, apocodeine, and nicotine*

In two experiments, XXIII and XXIV, the same results by warming were obtained with the heart as before, after atropine and nicotine were applied directly. In Experiment XXV, atropine, apocodeine, and nicotine were used successively in 1 per cent solutions upon the heart tissue directly. Before any drugs were applied the heart rate was doubled (100 per cent) when the thermode with temperature of 38°C. was placed on the S-A junction. There was no increase at any other point with that temperature. With temperature of 50°C. at other points on the sinus an increase in rate ensued also. After atropine was applied the increase by means of 40 to 50°C. temperature at the junction was 38 per cent, while there was no increase in rate at any other point. After the apocodeine was applied temperature effects were obtained only at the junction. In one case an increase of 43 per cent was noted. The rate now became slower gradually from 48 to 36 per minute, and then the nicotine was applied. The heart rate then dropped to 30 per minute. The auricles were beating feebly. Warming (49°C.) the junction increased the rate up to 100 per cent, while doing the same at a few other points increased the rate only 20 per cent.

Temperature effects on auricles and ventricle. No point on auricles or ventricle could be found which upon being warmed produced a change in rate.

C. *Direct electrical stimulation*

On the heart in situ, or excised, a series of observations were carried out in which various spots were directly stimulated by an electrical current, and we found that the least minimal electrical stimulus that will produce stoppage in a certain area will not do so at any other point. In this way, we often corroborated the results obtained by the temperature method. It was also determined that the strength of current is important in making a selective localization, because there is a tendency for a current to spread, and stoppage will then be obtained at a number of points. Figure 8 taken from Experiment XX is an example of this phenomenon.

In Experiment XXIV, direct electrical stimulation produced no effect after atropine and nicotine were applied directly to the sinus.

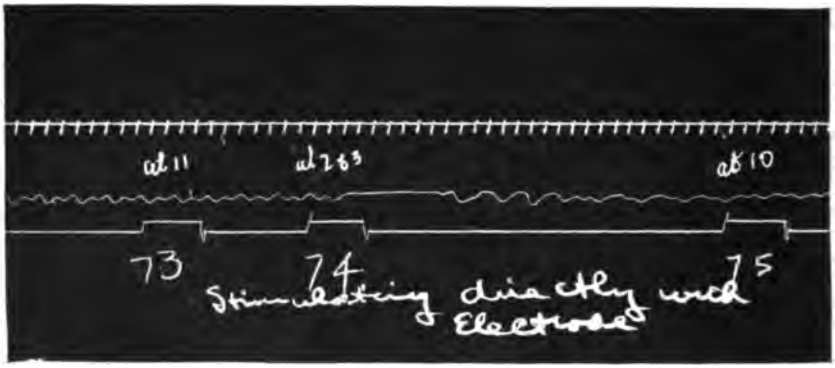

Fig. 8. For description see text

D. *Cauterization*

When a heated iron tip was applied to the auricle (Experiment XXI) there was at once a temporary increase in the rate due to the heating of the auricular tissue and the sinus adjacent, but when the effective spot was scalded, or seared, the heart became irregular, and its irregularity was far more evident with further scalding (Nos. 31, 46). With completer destruction of this spot the heart became very slow, thus simulating greatly the phenomenon that occurs with removal of the pacemaker from the upper parts of the specialized tissue in the mammalian heart, and the assumption of primary automaticity at some lower point in the specialized tissue.

DISCUSSION

1. *Temperature influences.* In the historical review above, it is noted as "well known that the automaticity of the heart, measured by its rate of stimulus production varies with the temperature . . . This influence of change of temperature is confined to that region

which is at the same time initiating the impulse or acting as 'pacemaker' for the whole heart" (8).

The principle laid down by Gaskell and Engelmann and supported by Hering, that the most automatic part of the heart is the pacemaker at that given moment, may explain the slowing produced in the turtle's heart by cooling. Other points in the heart have less automaticity and initiate slower rhythms, therefore, when the primal spot is cooled, it is depressed, and primary automaticity is assumed at some other, and lower point. Each part of the specialized tissue probably has its own maximum and minimum rate, so that it is also possible to cool and warm the dominant spot, just enough, without having the pacemaker change its position.

Our results show that a distinction must be made as to the degree of temperature used. The results are quite different at 35 to 40°C. than at 45 to 55°C. At the lower range only one effective point could be found, while a number of them could be found at the higher range. In both ranges the dominant spot revealed itself by being affected immediately. At other points the change set in after a latent period, which is in agreement with Engelmann's (6) observations on the frog. He heated by means of a galvano-cautery, specifying that he did not cauterize the tissues. This makes it impossible to say what temperature was used, but our observations lead us to surmise that he obtained his results by a temperature which was in our higher range or even above. He states that the pacemaking function was assumed after a latent period by a vein after intenser warming. We have found the same to be true, and as a rule it was the right vein. MacWilliam (1) had previously noted that in the hearts of cold-blooded animals there is abundant evidence of high spontaneous rhythmic power constantly held in abeyance, and that the rhythm of the portion possessing higher spontaneous rhythm will supersede the inherent rhythm of the other parts and determine the rate of contraction in the whole organ. We believe that intense warming at practically any spot in the sinus and veins, as stated by Engelmann, will result in its assuming the pacemaking function because of the fact that the warming makes the tissue more automatic. Such a shift in pacemaking function by the temperature method has been demonstrated in the mammalian heart, and it has been shown that the pacemaker remained in some part of the specialized tissue. In the mammalian heart none of the workers have found points on the vena cavae where stimuli, especially thermic, could produce typical changes in heart activity, while they have again and again noted that the S-A junction, especially the node, was a most sensitive area, at which point heart activity could be influenced most surely, quickly, and typically.

The higher degree of temperature must have a much more striking influence in the turtle than in the mammal. The body temperature of our turtles varied from 18 to 20°C., while the highest temperature used on them varied from 45 to 55°C., giving a temperature difference of 27 to 35°C. The body temperatures of the mammals under experimental conditions were probably about 37°C. or possibly a little lower. Ganter and Zahn used a thermode at 50 to 60°C. (Adam 40 to 60°C.) which gives a temperature difference of 13 to 23°C. The highest difference used on the mammal is lower than the lowest difference for the turtle. Such a difference is important from the standpoint of the amount of heat that can be absorbed by the tissue in a given time. This may possibly explain why no typical results, if any at all, were obtained in the mammal on the veins; and probably results will be obtained if much higher temperatures or longer intervals of warming are used on the remnants of the sinus venosus, especially the termination of the superior vena cava.

Whether the difference produced by the two ranges in temperature is due to the fact that the tissue which can be influenced comes closer to the surface in the S-A junction as compared to other regions, we do not know. We doubt whether such a criticism could be maintained when the fact is considered that the tissue in the sinus and the veins is so thin, and ought to be easily influenced if there were no special mechanism for the initiation of the impulse. The problem of change in position of the pacemaker must be investigated in another way, at present best by the electronegative method.

Our results with the wider range of temperature indicate that a new seat of impulse formation in the region heated is produced by increasing the automaticity in this region. In the location of a cardiac pacemaker by warming it would seem to be important that the temperature range be small.

2. *Contractility and rhythmicity.* Engelmann emphasizes the fact that the veins had the power of contractility to a high degree, and that their power of rhythmicity was similar to that of the sinus. We believe that the ranges in temperature have revealed differences in rhythmicity The S-A junction which probably does not contract at all is most rhythmical. In their observations on dying mammalian hearts by the electronegative method Eyster and Meek (9) say, "This

suggests a possible differentiation of the properties of automaticity and contractility in certain regions of the heart. Our results merely show that impulse initiation (automaticity) may be present to almost normal extent (as judged by the rate of impulse formation) in regions in which contractility is greatly reduced or possbily absent."

3. *Cauterization.* In regard to the slow rate of the heart after searing we assume that less automatic portions became effective. Lohmann (10) was the first to conclude that abolition of the primary pacemaker in the mammalian heart made the activity take place in the A-V boundary, and his work has been confirmed by a number of workers. They have noted that as long as the sinus node was not badly injured it could again temporarily take up the function of impulse formation. This is apparently what happened in Experiment XXI, where for periods the slow rhythm was replaced by a faster one, or when warming the remains of the sino-auricular nodal tissue the latter was made more sensitive, and losing its depression, again assumed automaticity. No stoppage of the heart occurred, as so often happens in the mammalian heart, but there is such a mass of special tissue present in the cold-blooded heart, in the shape of the entire sinus venosus especially, that it seems as if there would always be some bit that would remain effective.

4. *Electrical stimulation.* Attention has been called to the fact that a difference in results ensues when the strength of direct electrical stimulation is varied The fact that a minimal strength is effective in only one region, the S-A junction, is significant. Gaskell (10) ventured the assumption that, "The probability is great that the automatic rhythm of all parts of the heart can be inhibited by the vagus nerves, when those nerve fibers are stimulated which supply directly the muscular fibers upon which the automatic rhythm in question depends." Flack (11) in "An investigation of the sino-auricular node of mammalian heart" obtained marked inhibition of both auricles and ventricles with weak stimulation of the node, but at no other point. We have data, to be reported later, which seem to indicate that the vagal endings exert great influence at the S-A junction. The S-A node when functioning as pacemaker in the mammalian heart has been shown to be intimately related with the vagi, and this seems to be true in the cold-blooded heart also.

5. *Localization of the pacemaker after drug application.* MacWilliam and Engelmann obtained their results on atropinized as well as normal animals. Our results were identical after the use of solutions of atro-

pine, apocodeine, and nicotine (to eliminate nerve influences as far as possible), in that the pacemaker was still in the S-A junction. It was noted that a higher temperature was needed to get results, indicating that the tissue had been depressed by the drugs.

Conclusion. Erlanger in particular has voiced the view that the S-A node in the mammal cannot reasonably be considered as the seat of impulse formation unless the same were true of the homologous structure in the cold-blooded animal. The S-A junction in the cold-blooded forms has been shown to be mostly nodal tissue. Meek and Eyster (5) have shown, as we have also, that the cardiac impulse originates in this definitely localized position in the turtle's heart, so that there is no more a distinction between cold- and warm-blooded hearts from this standpoint. "All vertebrate forms seem to agree in having the pacemaker of the heart located at the sino-auricular junction in masses or nodes of specialized tissue" (5). The rationalism demanded by Erlanger and others has therefore been justified.

SUMMARY

1. (a) Slight warming or cooling of the right sino-auricular junction or the parts adjacent to it, usually on the dorsal aspect, will increase or decrease the rate of the heart respectively. This change is produced only at this spot with a temperature range of 35 to 40°C.

(b) With a temperature range of 45 to 55°C. a number of points on the sinus and veins could be influenced to produce a change in rate

(c) In localization of a primary pacemaker by the temperature method the temperature range should be close to normal.

2. The application of atropine, apocodeine, and nicotine does not abolish the influence of temperature at the junction.

3. Stimulating the junction directly by a minimal electrical current will slow or stop the heart—and only at the junction. A stronger current applied to a number of points in the sinus and veins will slow or stop the heart.

4. The primary pacemaker in the turtle's heart has been shown to be in a definitely localized portion, and not the whole sinus venosus.

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THE CONDITIONS OF PHYSIOLOGICAL CONDUCTION IN IRRITABLE TISSUES

III. ELECTROLYTIC LOCAL ACTION AS THE BASIS OF PROPAGATION OF THE EXCITATION-WAVE

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In my two recent papers on conduction[1] I have advanced the theory that the transmission of the excitation-state from the immediate site of activity to the adjoining resting areas is dependent on an *electrical local action* of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces (*e.g.*, of iron) in contact with an electrolyte solution. Under such conditions—as is now well established—local couples are formed between those adjoining portions of the metallic surface which differ in composition or physical condition and hence in solution-tension, one region, that of higher solution-tension, forming the local anode, the other the local cathode. These local areas are the seat of electrolytic effects; at the local anode (where the positive stream enters the solution) the metal undergoes solution and chemical alteration (rusting in the case of iron), while at the cathode hydrogen (or other cation of similarly low solution-tension) is usually evolved and alkali set free. This cathodal deposition of hydrogen may incidentally have a polarizing effect and limit the current, hence the favorability of free oxygen or other depolarizing agent to the corrosive process, which is thus essentially electrolytic in nature.[2] The existence of these local chemical effects may be most readily demonstrated in the case of iron by the use of the "ferroxyl" reagent of Walker;[3] this is a solution containing phenolphthalein and ferricyanide and thickened by gelatine to retard diffusion; when this reagent is applied to the surface of ordinary iron, the anodic areas turn blue (from iron ferricyanide) and the cathodic areas red (from the alkali set free). Such local areas are more numerous in non-homogeneous than in homogeneous specimens of iron; the reason why the former rust so much more readily thus becomes clear. Rusting is in fact due chiefly to the electrolytic action of local circuits; such circuits are naturally more numerous when the surface has an irregular composition, with regions of lower and higher solution-tension. This is why perfectly pure iron is so remarkably resistant to corrosion.[4] Other conditions than simple chemical purity may however enter as factors; thus regions of mechanical strain have usually higher solution-tension than unstrained regions,[5] hence local mechanical treatment or pressure may cause rusting in pure iron;[6] the peculiar condition known as "passivity" may also play a part.[7] The dependence of rusting on local electrolytic action is further shown by the rust-preventing effects of contact with metals of higher solution-tension than iron, such as zinc;[8] in this case the zinc forms the anode, the iron the cathode, and oxidation of the latter is prevented,—hence the use of zinc strips to protect boiler-tubes, propellers and other iron parts from corrosion. Conversely, contact with nobler metals accelerates the corrosion; in this case the iron is the anode or dissolving electrode; accordingly tin-plated or nickel-plated iron rapidly rusts wherever the outer coating is broken through.[9]

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[1] This Journal, 1914, xxxiv, 414; 1915, xxxvii, 348.

[2] This theory is originally due to Faraday and de la Rive: "The whole subject of corrosion is therefore an electrochemical one, and the rate of corrosion is simply a function of electromotive force and resistance of circuit" (Whitney: Journ. Amer. Chem. Soc., 1903, xxv, 394). The literature is very large; some of the more important recent papers are cited below. For a discussion of the part played by depolarizing agents (in relation especially to the practical problem of protecting iron from rust) *cf.* Walker and Lewis: Journ. Ind. and Engin. Chem., 1909, i, 754.

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[3] Walker, Cederholm, and Bent: Journ. Amer. Chem. Soc., 1907, xxix, 1251.

[4] *Cf.* Lambert and Thomson: Journ. Chem. Soc., 1910, xcvii, Trans., 2426; Dunstan and Hill: *Ibid.*, xcix, 1835; Lambert: *Ibid.*, 1912, ci, 2056; Walker: *Loc. cit.*

[5] *Cf.* Desch's Metallography (in Ramsay's series of Textb. of Phys. Chem.), 1913, 286 *seq.;* Spring: Bull. Acad. Roy. Belg., 1903, 1066; Lambert: *Loc. cit.*

[6] See the instances cited in Desch's Metallography; Lambert: *Loc. cit.* Cadmium is especially sensitive to mechanical treatment; rubbing the surface of a piece of this metal makes the latter the anode when compared with an untreated specimen.

[7] *Cf.* Mugdan: Zeitschr. f. Elektrochem., 1903, ix, 442; Dunstan and Hill: Journ. Chem. Soc., 1911, xcix, 1835; Lambert: *Loc. cit.;* Grave: Zeitschr. f. phys. Chem., 1911, lxxvii, 513; MacLeod-Brown: Chem. News, 1913, cvii, 14; Grube: Chem. News, 1913, cviii, 259, 271, 283.

[8] For experiments illustrating this effect *cf.* Dunstan, Jowett, and Goulding: Journ. Chem. Soc., 1905, lxxxvii, Trans., 1548.

It is obvious that these facts have a highly general bearing; they apply in greater or less degree to all metallic surfaces in contact with electrolytes; and since all such effects depend primarily on the electromotor properties of the surfaces—i.e., their ion-combining or ion-liberating power—they must apply also to other surfaces, non-metallic in character, which have analogous electromotor properties—in all probability to all phase-boundaries across which ionic transfer of electricity is possible.

The intimate bearing of these and similar facts upon the conditions existing at the surface-films of living cells is plain. In my second paper I compared the living irritable element with an iron wire covered by a homogeneous film of pure zinc and immersed in an electrolyte solution;[10] such a wire is in chemical and electrical equilibrium and shows no change. But any interruption of the zinc coating sets up a local iron-zinc couple, and chemical and electrical effects at once result. The case of the film-covered surface of mercury in hydrogen peroxide solution (in the rhythmical catalysis of Bredig) is essentially similar, and offers closer biological analogies; here the local electrolytic process initiated by the local interruption of the film causes the dissolution of the film-substance, and hence the effect is propagated over the whole surface of the mercury.[11] Similarly in the resting irritable element, *e.g.*, nerve-fibre, the unaltered plasma-membrane represents a homogeneous surface-film of uniform electromotor properties; the local stimulating agent causes locally a change in the electrical condition of the surface; the potential-difference between protoplasm and medium is decreased at that point (in consequence apparently of the increased permeability of the film), and a local couple arises, with the current flowing in the external medium from the unstimulated to the stimulated region. According to the hypothesis advanced in the paper cited, this local circuit, having once originated, produces effects similar to those of any other electrical current upon the adjoining portions of the irritable element; at the still resting regions the direction of the current entering the cell-surface from the external medium is such as to cause local depolarization,[12] an effect resulting, if sufficiently intense, in stimulation. This secondarily stimulated region produces similar effects at the region beyond; hence the excitation-effect *spreads*, at a rate proportional to the rate of development of the local current and the maximal distance (from the original area of excitation) through which its stimulating effect is felt.

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[9] The effect of contact of carefully purified iron with a noble metal (platinum) is well shown in experiments by W. A. Tilden: Journ. Chem. Soc., 1908, xciii, Trans., 1356.

[10] Loc. cit., 357.

[11] Antropoff: Zeitschr. f. phys. Chem., 1907, lxii, 513.

This theory, which I propose to call "the local action theory" of conduction, evidently assumes that the electromotor properties of the protoplasmic surface-film are determined by conditions which are fundamentally similar to those governing the electromotor phenomena at metallic surfaces. That this is actually the case is clearly indicated by the observations of Macdonald,[13] Loeb and Beutner,[14] and Beutner[15] on living cells, organic membranes, and solutions of lipoids in organic solvents. The surfaces of such systems behave as if reversible to cations as a class, and the law of Nernst applies; any such surface must therefore be regarded as possessing, like a metal, an electrolytic solution-tension,—one, however, which presumably varies with the physical and chemical condition of the surface-film.[16] Variations in the solution-tension of such a protoplasmic surface-film would produce essentially the same electrical effects as variations in the solution-tension of a metallic surface. Local alteration of the cell-surface would thus give rise to local electrical circuits; these, like other currents, would exert stimulating and inhibiting influences on adjacent portions of the cell, or possibly on neighboring cells. In the present paper I wish to pursue this comparison in further detail, and to call attention to certain other analogies between the phenomena of local action at metallic surfaces and at the surfaces of living cells.

In the irritable living element the local effect differs from that in the metal in being *evanescent*, and in being propagated quickly from the original site of disturbance; *i.e.*, the change at the excitation-area is there immediately reversed, and is transmitted with greater or less rapidity to adjoining regions; in other words, it *spreads*, at a rate determined by the temperature, the composition of the medium, and the specific peculiarities of the tissue. The problem of the physico-chemical basis of this "conduction" requires for its solution a determination of the general conditions under which surface-changes of this kind spread from active to inactive regions, whether in a living cell or an inorganic system.

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[12] *Cf.* p. 362 of my paper cited.

[13] Macdonald: Proc. Roy. Soc., 1900, lxvii, 325.

[14] Loeb and Beutner: Biochem. Zeitschr., 1912, xli, 1; xliv, 303; 1913, li, 288.

[15] Beutner: Zeitschr. f. Elektrochem., 1913, xix, 319, 467; Trans. Amer. Electrochem. Soc., 1913, xxiii, 401; this Journal, 1913, xxxi, 343.

[16] This, however, is also true for metals; witness the difference between active and passive iron, the two varieties of tin or chromium, strained and unstrained metals.

It is first to be noted that spreading effects, showing in many ways close analogies with those of living tissues, are frequently met with in simple inorganic systems of the above class. The case of the film-covered mercury-surface in hydrogen peroxide solution is an instance which I have already cited in former papers.[17] Other interesting analogies are seen in the spreading of the "active" condition over the surface of "passive" iron or other similar metal in consequence of a local alteration which may, as in the case of living tissues, be of a mechanical, chemical, or electrical kind. The passivity is thus locally destroyed, and the effect spreads over the whole surface. This phenomenon is thus described by Dunstan and Hill.[18] Iron in the passive state differs from normal iron in not being attacked by nitric acid (s.g.1.2), but remains bright and unaffected in the solution; it is however at once attacked if touched with a piece of active iron, or scratched with a glass rod; the action then visibly spreads from the point of contact over the whole surface. Similarly a solution of $CuSO_4$ (0.5 per cent) deposits a film of copper on active iron, but not on passive iron, which remains bright until touched with active iron or scratched; the film of copper can then be seen to spread from the region of contact. A mechanical jar is often sufficient to activate a piece of passive iron, or local bending or pressure;[19] electrical currents, especially if of high density, may have a similar effect.[20] In all of these effects the destruction of passivity and the transmission of the active condition are almost certainly due to an alteration of solution-tension and the formation of local electrolytic couples. Passive iron has a lower electrolytic solution-pressure than active iron.[21] The activated region thus becomes the anode in the local circuit; the region still passive is the cathode; the current thus passing alters the condition at the temporary cathodal areas which are thus rendered active—possibly by the nascent hydrogen there freed—and so the effect spreads until the whole surface is activated. The transmission of activation in metals is thus due to electrical conditions,—just as in the living system, according to the present theory. Both effects may in fact be regarded as autocatalyses of a special kind.

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[17] This Journal, 1909, xxiv, 18; 1914, xxxiv, 426; Popular Sci. Monthly, 1913, 151.

[18] Dunstan and Hill: Journ. Chem. Soc., 1911, xcix, Trans., 1835, 1853. See also Lambert's description of the behavior of pure iron in solutions of copper salts; copper is only slowly deposited on pure iron from dilute $CuCl_2$ solutions; dull spots appear on the bright surface of the iron and the effect spreads quickly (p. 2070). Similarly spots of rust on a bright iron surface tend to spread, the deposit having, as it were, an autocatalytic influence. The rusted region acts as cathode relatively to the unrusted regions which, as anodes, tend to dissolve, etc.

[19] *Cf.* Dunstan and Hill: *Loc. cit.;* also Lambert: *Loc. cit.*

[20] *Cf.* Hayden: Journ. Franklin Inst., 1911, 295. Active chromium is rendered passive by having a strong current pass through it as anode (Le Blanc: Zeitschr. f. Elektrochem., 1900, vi, 472). The same is true of nickel, and apparently also of iron (Le Blanc: Boltzmann Festschr., 183).

This brief description does not pretend to discuss the phenomena of activation and passivation in metals in any detail, but merely to call attention to the more important biological analogies. The spreading of the effects of stimulation or other local alteration is one of the most characteristic peculiarities of living systems; it is in fact essential to any form of stimulation. In any such process a propagated disturbance[22] is set up by some form of local alteration; in this way the whole cell or element is activated. The local action theory attributes this effect to the formation of a local circuit at the original site of alteration; by the action of this circuit the altered condition is automatically transmitted to adjoining regions. Some of the most striking recent examples of such transmission are afforded by the observations of Kite and Chambers on the effects following puncture or tearing of the surface-layers of various cells by microscopic glass needles. A leucocyte or red blood corpuscle thus locally injured undergoes rapid and complete dissolution;[23] many other cells show similar changes;[24] the effect of the local injury spreads from the altered region over the entire cell-surface; sometimes the entire cell goes into solution with almost explosive rapidity.[25] It is clear that the local injury initiates a disturbance which is rapidly propagated and results in the destruction of the osmotic properties of the whole surface-film; Chambers observes that when a red corpuscle is punctured the haemoglobin diffuses out; "it is significant that the outflow of the haemoglobin does not take place only at the punctured spot, but over the entire surface of the corpuscle."[26] In such phenomena we have instances of transmission of an altered condition of the surface-film—involving evidently a marked increase of permeability—which are probably identical in their general characters with the transmission of the normal excitation-state in irritable elements. Both phenomena represent special instances of protoplasmic conduction, although in the one case reversible, in the other irreversible, effects are produced. The analogies to the transmission of the altered state of the metallic surface in the above cited experiments of Dunstan and Hill are evident. In both cases we have to do with "spreading phenomena" initiated by a local alteration; in the case of the metal the effect is clearly due to electrical circuits resulting from local changes in the electromotor properties of the surface; the same is the case, according to the present theory, in the transmission of the effects of local alteration of the cell-surface.

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[21] LeBlanc: Boltzmann Festschr., 1904, 183; Mugdan: Zeitschr. f. Elektrochem., 1903, ix, 442; Grave: Zeitschr. f. phys. Chem., 1911, lxxvii, 513; MacLeod-Brown: Chem. News, 1913, cvii, 14; Grube: Chem. News, 1913, cviii, 259, 271, 283.

[22] This term of Keith Lucas (Journ. Physiol., 1912, xliv, 68) is applicable not only to the transmission of the excitation-state, but also to the spreading of the effects of local injury in cells (about to be described), as well as to the propagation of the active state over the surface of passive metals.

[23] Chambers: Lancet-Clinic, March 27, 1915; *cf.* also Oliver: Sci., 1914, N. S., xl, 645.

[24] Kite describes such effects for Amœba and Paramœcium (this Journal, 1913, xxxii, 146); Chambers for the germ cells of insects (Sci., 1914, N. S., xl, 824; 1915, xli, 290).

In the case of metals the conditions determining the rate at which the activation-effect is transmitted have not yet been fully ascertained; but it is evident that since the local electrolytic process is the primary feature of the whole phenomenon, the rate of this process must determine the rate of transmission. The velocity of the electrolytic process in any such local couple (or in a galvanic cell of any kind) depends (1) upon the potential-difference between the anodic and cathodic areas (*i.e.*, the difference between the electrolytic solution-pressures at the two regions), and (2) upon the electrical resistance of the circuit. Ericson-Aurén and Palmaer give the formula, $V = f \frac{x}{C} e$, where V is the velocity of solution of the metal (*e.g.*, iron) at the local anode, f a proportionality factor, x the conductivity of the adjoining solution, e the potential-difference, and C the resistance-capacity of the arrangement (*i.e.*, the resistance attained while the reaction is proceeding[27]). If, as we must assume, the local electrolytic process has to reach a certain stage before the effect is transmitted to adjoining areas, the rate of this transmission will also depend upon the same conditions,—*i.e.*, will be proportional to the rate of the local action. This explains why the transmission of the active state, as well as the rate of corrosion in general, is more rapid in electrolyte solutions than in pure water.[28]

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[25] *Cf.* Kite: Loc. cit., 156; Chambers: Loc. cit., 1915.

[26] Chambers: Proc. Amer. Assoc. Anatomists (abstract in Anat. Record, 1916, x, 190).

In a conducting tissue like nerve analogous conditions must be presumed to enter. The intensity of the local effect will depend upon the potential-difference (equivalent to difference of electrolytic solution-tension) between the active and the inactive areas, and upon the electrical conductivity of the circuit. As I pointed out in my last paper, the intensity of the current traversing the cell-surface at a point *R*, distant by the interval *SR* from the original point of stimulation *S* (which corresponds to the local cathode in the wire model), is dependent on the resistance of this part of the circuit.[29] Hence if a certain critical intensity of current is required for local stimulation at any point, the rate at which the excitation-effect is transmitted from the excited region *S* to an adjacent unexcited region *R* should depend upon the electrical resistance of the circuit formed by the intervening stretch of irritable element and the medium bathing it. A relation of direct proportionality should thus exist between the electrical conductivity of the medium and the rate of propagation of the excitation-wave. Whether or not such a relation is general in conducting tissues has not yet been systematically investigated. The recent observations of Mayer,[30] however, on the rate of conduction in the nerve-net of the medusa Cassiopea in dilute sea-water, seem to exemplify clearly just this kind of relationship—although not interpreted by Mayer himself in this sense. The parallelism between the rate of propagation of the contraction-wave and the electrical conductivity of the medium (with dilutions ranging from 5 to 50 volumes per cent distilled water) is remarkably close. In any such investigation a tissue whose properties are not essentially altered by considerable changes in the osmotic pressure of the medium is needed, and the physiological balance of the salts in the medium should remain unchanged. These conditions are apparently met in Mayer's experiments; and it seems highly probable that the diminished rate of physiological conduction observed by him in dilute sea-water is a direct consequence of the diminished electrical conductivity of the medium and of the protoplasm.[31] This is the result which the local action theory of conduction would lead us to expect. Further investigation of this relationship in a variety of conducting tissues is however needed, and I hope before long to be able to present further data on this subject.

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[27] Ericson-Aurén and Palmaer: Zeitschr. f. phys. Chem., 1901, xxxix, 1. *Cf.* also the other papers of these authors: *Ibid.*, 1903, xlv, 182, and 1906, lvi, 689.

[28] *Cf.* Dunstan and Hill: Loc. cit., 1856. For the relation between the conductivity of the medium and the rate of corrosion *cf.* also Ericson-Aurén and Palmaer, Whitney, Lambert and Walker in the papers above cited.

[29] Loc. cit., 360.

[30] A. G. Mayer: Proc. Nat. Acad. Sci., 1915, i, 270 and 1916, ii, 37; this Journal, 1916, xxxix, 375.

In the transmission of the state of excitation from the active to the adjoining inactive regions in a conducting element like a nerve-fibre, the conditions appear to be essentially the same as in the spreading of the altered condition of the surface-film in the above cited experiments of Kite and Chambers. The two processes differ however in that the rate of transmission in the irritable element is usually much higher, and the effect at the active region is *transitory*, *i.e.*, the changes associated with excitation (negativity and increased permeability) are rapidly and automatically reversed when the wave of excitation reaches any region of the element. Thus in the typical irritable element like nerve the local excitation-process is automatically self-limiting. The high velocity of transmission is apparently a function of high irritability—*i.e.*, promptness and rapidity of local change, and great sensitivity to variations of surface-polarization—and its conditions have been discussed in the first paper of this series. With regard to the self-limiting characteristic, there is every indication that this is at bottom nothing but an expression of the well-known polar action of the electrical current upon such tissues,—*i.e.*, inhibition where the current enters the element (at the anode of the electrodes from an external source of current), and stimulation where it leaves. As shown in the diagram on page 362 of my last paper, the current of the local active-inactive circuit has such a direction that at the original site of excitation the positive stream *enters* the cell-surface from the medium, while at adjoining regions (not yet excited) it *leaves*. Since there is no reason to believe that a self-generated current will have different effects upon a tissue from one reaching the latter from outside, we must conclude that the local current, having once originated, *itself* inhibits the excitation at the active area, in addition to initiating excitation at the adjoining inactive areas. This process is automatically repeated at the new region of excitation, and so the effect is transmitted as a wave over the whole element. The present hypothesis therefore simply assumes that the action-currents themselves influence the activity of the tissues by which they are generated—and also probably that of adjacent cells and tissues[32]—and that this influence is of the same nature as that exerted by electrical currents in general, whatever their source. If this is granted, the immediate inhibition of activity at the original site of activity, and its initiation at the adjoining regions, will be seen to be automatic and necessary consequences of local activity in any irritable element having the properties of a nerve-fibre. Any local electrical circuit in a system with such properties will of necessity locally limit itself and be transmitted to adjacent regions. Hence a wave of electromotor variation travels along the surface of the irritable element.

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[31] Mayer believes that the rate of nerve conduction is proportional to the quantity of ions *adsorbed*, rather than to the concentration of ions in solution. In the formula which he finds best to agree with his observations ($y = 2.512\ x^{0.8}$, y being the rate of conduction, and x the concentration of Na, Ca and K ions in solution in the medium), the assumed adsorption exponent, 0.8, is so near unity that the assumption of a direct proportionality between the rate of conduction and the concentration of ions in solution—*i.e.*, the electrical conductivity—gives an almost equally good agreement with observation. Possibly the dilute sea-water may somewhat impair the tissue and account for the slight deviation from direct proportionality with moderate dilutions; at dilutions above 50 per cent the rate of conduction falls off much more rapidly than the electrical conductivity.

It may be pointed out that the need for salts in the protoplasm and media of irritable tissues is in all probability largely an expression of this need for electrical conductivity (*cf.* the first paper of this series, this Journal, 1914, xxxiv, 429).

It should be noted that this self-limiting effect is not an unexampled and distinctive peculiarity of living tissues, but is shown to a greater or less degree by all circuits having polarizable electrodes. A counter electromotive force is set up as the current starts, which diminishes the latter, to a degree and at a rate which depend on the special nature of the conditions. Thus in the case of a circuit of copper and platinum in a solution of $CuSO_4$ the electromotive force gradually falls to zero owing to the deposition of copper on the platinum.[33] In the active nerve-fibre or similar irritable system we must assume that in an analogous manner some process occurs where the current enters the cell-surface—which arrests activity and restores the resting properties of the surface-film, *i.e.*, causes re-polarization; while at the region where the current leaves, the reverse process occurs, and the film undergoes an alteration involving increased permeability and change of electromotor properties; this change, however, is immediately reversed in the manner already indicated. The precise metabolic basis of these changes cannot be indicated at present; the above facts, however, imply that processes of an essentially electrolytic character play a fundamental part.

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[32] For example, it is possible that such a phenomenon as the reciprocal inhibition of antagonistic neurones in the central nervous system may be due to these conditions, as I pointed out in a former paper (this Journal, 1913, xxxi, 285). It may be that the bioelectric processes have a general coördinating influence of still wider application (*cf.* the first paper of this series, *loc. cit.*, 427 *seq.*).

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[33] It should be noted that living tissues in general exhibit a high degree of electrical polarizability; polarization-currents of a strength sufficient for stimulation arise immediately when a current is passed through such a tissue.

CRYOSCOPIC MEASUREMENT OF THE OSMOTIC DIFFERENCE BETWEEN RESTING AND FATIGUED MUSCLE

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It has been shown by Ranke (1) that when striated muscle is fatigued and put into an indifferent salt solution, it absorbs water in greater measure than a resting muscle kept in the same solution. Ranke sought to account for this increased inbibition on the part of fatigued muscle by supposing dissociation products to be formed in the tissue as the result of its activity, and this to bring about an increased attraction for water. In modern terms, fatigue increases the osmotic pressure of muscle by breaking down some of the larger molecules into more numerous smaller ones.

Other workers have confirmed Ranke's results. The method consists in immersing the muscle in a solution of known concentration from which it is removed from time to time and weighed. In this way Cooke (2) showed that the increase in osmotic pressure may be as high as 37.5 per cent as the result of fatigue. Later, Fletcher (3) found the curves of swelling of resting and of fatigued muscle to differ characteristically, the latter always reaching an earlier maximum weight. In salt solutions isosmotic with the animal's blood the fatigued muscle gained in weight but the normal control remained unchanged for hours.

In case the muscle be exercised in the body with the circulation intact, it increases in weight (4), volume (5) and decreases in specific gravity (6) indicating water absorption from the lymph. It is known that the blood flowing from an exercising muscle suffers a loss in water and an increase in solid content (7). It has been suggested by certain workers that the increased imbibition of water by an exercised muscle may be due in part to changes in the permeability of the muscle cells (8). The connection between the acidity accompanying muscle contraction and water absorption has been frequently emphasized, and by some, acidity is considered a possible cause of muscle contraction (9).

The views referred to are sufficient to indicate the unsettled condition of the question of water absorption by muscle, and the importance of obtaining new evidence on the point at issue, (10), viz: Is the increase of osmotic pressure in a muscle, resulting from fatigue, alone sufficient to account for the increased imbibition on the part of such a muscle? It is apparent that measurements of weight increment and of specific gravity show the fact of water absorption solely, without indicating unequivocally the underlying cause. In order to determine exactly what part osmotic pressure plays in causing the phenomenon in question, it is necessary to measure the concentration of dissolved substances in the muscle, both resting and fatigued, and to compare the values obtained. Cryoscopic determinations afford a reliable method for doing this, because, in dilute aqueous solutions such measurements depend entirely upon the gram-molecular concentration and, in the case of electrolytes, the degree of ionization.

Fifteen years ago Sabbatani (11) showed that the freezing point of uninjured tissue could be determined with a fair degree of accuracy. This was done by packing the tissue around the bulb of a Beckmann's thermometer, allowing the preparation to undercool until spontaneous crystallization took place, and reading, as the freezing point, the highest mark reached by the mercury column. I have found it possible by the use of this method, to duplicate my observations with an experimental error of ±0.01°, provided the degree of undercooling be controlled by inoculating the preparation with an ice crystal.

In the following experiments large bull frogs, *Rana catesbiana*, were used. The animal was killed and the muscles of one leg removed with as little injury as possible. The superfluous liquid was absorbed from the tissue with filter paper, and the muscles packed around the bulb of the thermometer in a Beckmann's apparatus. Two or three successive readings were taken. The highest reading of each experiment, i.e., the least depression, is given in the table. Meantime the other leg of the same animal was prepared by removing the skin and absorbing the superfluous lymph with filter paper. The preparation was next placed in a moist chamber and stimulated directly with a maximal tetanizing current until completely fatigued, usually about two hours. It was then removed and the freezing point of the muscles determined as described for the other leg. Since the loss in weight of the preparation during stimulation was less than 0.5 per cent, any appreciable lowering of the freezing point below that of the control must be attributed alone to an increase in the concentration of dissolved substances or their dissociation products, in the fatigued tissue. The following measurements show that in every case the freezing point of frog's muscle is significantly depressed by fatigue (table 1). Each numeral indicates a pair of measurements made from a single individual. Δr = depression of the freezing point for resting muscle; Δf = that for fatigued muscle; $\Delta f - \Delta r$ = increase in depression of the freezing point due to fatigue; M = gram-molecular concentration of a non-electrolyte sufficient to produce the increase $\Delta f - \Delta r$, hence $M = \frac{\Delta f - \Delta r}{1.85}$, in which 1.85°C. = depression of the freezing point of a molar solution; per cent = per cent of increase in the depression of the freezing point resulting from fatigue.

These results show that the increase in the osmotic pressure of muscle due to fatigue amounts to an average of 36 per cent which is equivalent to an increase in concentration of 0.08 mol and is of sufficient magnitude to account for the imbibition of water previously observed.

TABLE 1

	Δr –°C.	Δf –°C.	$\Delta f - \Delta r$ –°C.	M	PER CENT
1	0.43	0.54	0.11	0.06	26
2	0.43	0.56	0.13	0.07	30
3	0.41	0.54	0.13	0.07	32
4	0.385	0.545	0.16	0.085	42
5	0.41	0.565	0.155	0.085	38
6	0.40	0.58	0.18	0.095	45
7	0.405	0.58	0.175	0.095	43
8	0.39	0.57	0.18	0.095	46
9	0.41	0.55	0.14	0.075	34
10	0.41	0.58	0.17	0.09	41
11	0.43	0.58	0.15	0.08	35
12	0.42	0.56	0.14	0.075	33
13	0.45	0.56	0.11	0.06	24
14	0.43	0.57	0.14	0.075	33
15	0.45	0.62	0.17	0.09	38
16	0.46	0.63	0.17	0.09	37
Mean.........	0.42	0.57	0.15	0.08	36

The increase in water absorption from a surrounding solution by a fatigued muscle is therefore brought about by an increase in the concentration either of molecules or of ions or of both, within the tissue.

The experiments were repeated with the circulation intact. This was best accomplished by destroying the brain anterior to the medulla, and severing the sciatic plexus from the cord on both sides. This section was performed through an opening into the body cavity made by removing the urostyle. A frog prepared in this way retained its respiration and circulation. The entire animal was put into a moist chamber and one leg was fatigued by direct stimulation. The freezing points of both resting and fatigued muscles were determined and found to be identical as shown in table 2.

TABLE 2

	Δ_r −°C.	Δ_f −°C.	$\Delta_f - \Delta_r$ −°C.
1	0.47	0.46	−0.01
2	0.46	0.46	0.00

This result agrees with the observations of Lazarus-Barlow, Barcroft and others who have shown that a muscle, which is exercised *in situ* with the circulation intact, compensates in part for its hypertonicity by absorbing water from the lymph and ultimately from the blood. If we further suppose the circulation to remove some of the fatigue products, the identity of freezing points of resting and of fatigued muscles is to be expected.

CONCLUSIONS

1. The mean depression of the freezing point for the resting muscle of the bull frog was found to be 0.42°C.
2. The mean depression of the freezing point for the completely fatigued muscle of the bull frog was found to be 0.57°C.
3. The mean difference in freezing points for resting and fatigued muscle is 0.15°C. This is equivalent to the depression of the freezing point of an 0.08 M solution of a non-electrolyte.
4. On account of the magnitude of the increase in the depression of the freezing point as a result of fatigue, it is concluded that the increased imbibition of fatigued muscle is caused entirely by the increase in concentration of dissolved substances, ions and molecules, within the muscle.
5. The freezing point of muscle fatigued in the body with circulation intact is identical to that of the resting muscle of the same individual.

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SOME METABOLIC EFFECTS OF BATHING IN THE GREAT SALT LAKE. II

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In a previous paper (1) it was shown that bathing in the strong brine of Great Salt Lake increased the urinary elimination of nitrogen 5 to 7 per cent, and that of sodium chloride 21 to 27 per cent in two subjects on a uniform diet. To determine whether these findings are significant and constant, and whether they are causally related, a second experiment was carried out along similar lines on the same subjects. The uniform diet was as follows:

	MORNING		NOON AND EVENING	
	Subject 1	Subject 2	Subject 1	Subject 2
Toasted wheat biscuit	30 grams	30 grams		
Strawberries	50 grams	50 grams		
Graham crackers	90 grams	60 grams	120 grams	90 grams
Butter	25 grams	20 grams	25 grams	25 grams
Peanut butter	20 grams	10 grams	20 grams	10 grams
Sugar	5 grams	5 grams		
Milk	465 cc.	465 cc.	465 cc.	465 cc.

Protein per day (calculated), Subject 1, 102 grams; Subject 2, 85 grams.
Calories per day (calculated), Subject 1, 3455; Subject 2, 2857.

The experiment was divided into three periods; (1) a fore period of five days, (2) a bathing period of four days, and (3) a final period of three days. The urine was collected in 24-hour samples on six of the days and in three to four hour samples on the other six days. Of these latter samples 24-hour composites were also made. Analyses were made for total nitrogen (Kjeldahl), ammonia (Folin), creatinine (Folin), uric acid (Benedict-Hitchcock) and chlorides (Dehn-Clark). All the determinations were in duplicate except those of total nitrogen and chlorides on the short period samples.

The variation in the urine constituents is shown graphically in figure 1, and the analytical data from which the figure was made, with record of temperature and length of baths, are given in table 1. In making the averages for the fore period the data of the first two days were discarded since they were influenced by the preceding mixed diet and by small changes in the uniform diet which were found necessary for the satisfaction of the subjects. The data of the period samples, taken on days 5 to 7 and 9 to 11 are given in tables 2 and 3, and are shown graphically in figure 2. Of these six days, 6, 7 and 9 are bathing days. In the figure the time of the bath and its duration are indicated by the line *B*.[1]

Referring first to the data in figure 1 and table 1 it appears that the average daily total nitrogen excretion increases slightly in the bathing period over the fore period, which is in agreement with previous findings. There is a gradually decreasing nitrogen output during the fore period and a gradually increasing nitrogen output in the bathing period. The maximum is reached on the day following the last bath, in both subjects, and in the final period the average daily nitrogen excretion exceeds that of either of the preceding periods, i.e., the high level reached at the end of the bathing period is practically maintained in the final period, a result differing from previous findings.

The other nitrogenous urinary constituents show so little variation as to permit of no conclusions. The very slight rise in creatinine excretion noted in the bathing period of the former experiment is not observed here.

In contrast to the results of the former experiment in which the average excretion of sodium chloride was increased during the bathing period there is shown in the present data a decreased output of sodium chloride during the bathing period, as compared with the fore period, while in the final period the average output is above that of the fore period.

The excretion of nitrogen and of chlorides in the case of both subjects shows a striking uniformity throughout, a progressively decreas-

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[1] It is perhaps unfortunate that absolute uniformity was not observed in the duration of these periods but the value of the date is not materially diminished on this account.

TABLE 1

Subject 1

DAY OF EXPERIMENT	DATE	TOTAL NITROGEN	AMMONIA	URIC ACID	CREATININE	SODIUM CHLORIDE	TEMPERATURE OF Air	TEMPERATURE OF Water	LENGTH OF BATH
		grams	*grams*	*grams*	*grams*	*grams*			*min.*
1	June 3	13.79	0.47	0.55	1.24	8.75			
2	June 4	13.23	0.37	0.55	1.30	9.02			
3	June 5	12.58	0.33	0.65	1.24	9.82			
4	June 6	12.15	0.34	0.58	1.35	9.09			
5	June 7	11.90	0.32		1.40	6.87			
Average 3–5...		12.21	0.33		1.33	8.59			
6	June 8	12.47	0.29	0.55	1.32	6.59	25	24.5	25
7	June 9	12.56	0.31	0.39	1.29	7.50	24	24	40
8	June 10	12.72	0.30	0.42	1.29	8.40	23	24	45
9	June 11	13.56	0.32	0.41	1.31	8.68	24	26	45
Average.......		12.83	0.31	0.44	1.30	7.79			
10	June 12	13.70	0.32	0.41	1.27	8.83			
11	June 13	13.32	0.34	0.42	1.29	8.84			
12	June 14	13.49	0.31	0.45	1.31	8.64			
Average.......		13.50	0.32	0.43	1.29	8.77			
				Subject 2					
1	June 3	9.44	0.32	0.52	0.92	8.54			
2	June 4	9.93	0.29	0.56	0.91	8.16			
3	June 5	9.18	0.24	0.38	0.92	8.02			
4	June 6	9.53	0.30	0.36	0.99	7.40			
5	June 7	8.55	0.33		0.96	5.10			
Average 3–5...		9.09	0.30		0.94	6.84			
6	June 8	8.85	0.27	0.29	0.93	4.20	25	24.5	25
7	June 9	8.86	0.27	0.39	0.93	5.16	24	24	30
8	June 10	9.49	0.19	0.38	1.02	7.43	23	24	45
9	June 11	10.84	0.24	0.48	1.02	8.75	24	26	35
Average.......		9.51	0.24	0.39	0.98	6.39			
10	June 12	11.16	0.25	0.35	0.98	8.32			
11	June 13	10.91	0.28	0.39	0.99	8.79			
12	June 14	10.65	0.25	0.37	1.03	8.94			
Average.......		10.91	0.26	0.37	1.00	8.68			

TABLE 2

Subject 1

DAY OF EXPERIMENT	PERIOD	TOTAL NITROGEN		SODIUM CHLORIDE		CREATININE	
		Per period	Per hour	Per period	Per hour	Per period	Per hour
5 (no bath)	8.00–11.00	1.48	0.494	1.19	0.395	0.20	0.068
	11.00– 3.00	2.12	0.530	1.83	0.456	0.25	0.062
	3.00– 6.00	1.29	0.429	0.70	0.234	0.17	0.057
	6.00–10.00	2.46	0.614	1.26	0.314	0.24	0.060
	10.00– 8.00	4.30	0.430	1.95	0.195	0.49	0.049
	For 24 hours	11.64		6.90		1.36	
	Composite	11.90		6.87		1.41	
6 (bath)	8.00–11.00	1.51	0.504	0.83	0.278	0.18	0.059
	11.00– 4.00	2.81	0.562	1.72	0.345	0.29	0.059
	4.00– 7.00	1.96	0.653	1.47	0.491	0.18	0.059
	7.00–10.00	1.62	0.540	0.86	0.288	0.17	0.055
	10.00– 8.00	4.59	0.459	1.74	0.174	0.51	0.051
	For 24 hours	12.49		6.64		1.32	
	Composite	12.47		6.59		1.32	
7 (bath)	8.00–12.00	1.68	0.421	1.07	0.267	0.21	0.053
	12.00– 3.30	2.00	0.571	1.37	0.392	0.21	0.059
	3.30– 6.30	2.13	0.709	1.98	0.661	0.18	0.060
	6.30– 9.30	1.76	0.586	0.90	0.298	0.17	0.057
	9.30– 8.00	5.00	0.476	2.07	0.198	0.50	0.048
	For 24 hours	12.57		7.39		1.27	
	Composite	12.56		7.50		1.29	
9 (bath)	8.00–12.35	2.31	0.505	1.75	0.380	0.27	0.060
	12.35– 4.20	2.34	0.625	1.62	0.431	0.22	0.059
	4.20– 7.35	2.48	0.762	2.58	0.793	0.20	0.061
	7.35–11.15	2.14	0.584	1.21	0.331	0.19	0.053
	11.15– 8.00	4.37	0.499	1.55	0.177	0.43	0.050
	For 24 hours	13.64		8.71		1.32	
	Composite	13.52		8.68		1.31	
10 (no bath)	8.00–11.40	2.02	0.552	1.53	0.418	0.21	0.058
	11.40– 3.40	2.58	0.644	2.18	0.544	0.24	0.060
	3.40– 6.45	2.00	0.649	1.29	0.419	0.18	0.057
	6.45–11.30	3.10	0.653	2.25	0.474	0.27	0.057
	11.30– 8.00	4.04	0.476	1.55	0.183	0.38	0.045
	For 24 hours	13.75		8.80		1.28	
	Composite	13.70		8.83		1.27	
11 (no bath)	8.00– 1.30	3.24	0.589	3.01	0.547	0.35	0.064
	1.30– 4.30	1.91	0.638	1.48	0.492	0.17	0.058
	4.30– 7.15	1.57	0.571	1.16	0.420	0.15	0.055
	7.15–11.15	2.59	0.646	1.52	0.380	0.23	0.058
	11.15– 8.00	4.03	0.461	1.68	0.192	0.41	0.047
	For 24 hours	13.34		8.84		1.31	
	Composite	13.31		8.84		1.29	

TABLE 3

Subject 2

DAY OF EXPERIMENT	PERIOD	TOTAL NITROGEN		SODIUM CHLORIDE		CREATININE	
		Per period	Per hour	Per period	Per hour	Per period	Per hour
5 (no bath)	8.00–11.00	1.14	0.381	1.12	0.371	0.14	0.048
	11.00– 3.00	1.50	0.376	1.42	0.356	0.17	0.043
	3.00– 6.00	0.80	0.267	0.58	0.192	0.12	0.040
	6.00–10.00			0.95	0.238	0.17	0.043
	10.00– 8.00	3.39	0.339	1.12	0.112	0.36	0.036
	For 24 hours			5.18		0.97	
	Composite	8.55		5.10		0.96	
6 (bath)	8.00–12.00	1.41	0.352	0.82	0.205	0.17	0.042
	12.00– 4.00	2.09	0.523	0.91	0.227	0.18	0.044
	4.00– 7.00	1.28	0.427	0.77	0.257	0.13	0.042
	7.00–10.00	1.12	0.372	0.49	0.163	0.11	0.038
	10.00– 8.00	3.35	0.335	1.23	0.123	0.38	0.038
	For 24 hours	9.24		4.22		0.97	
	Composite	8.85		4.20		0.93	
7 (bath)	8.00–12.00	1.18	0.296	0.86	0.214	0.16	0.040
	12.00– 3.30	1.44	0.411	1.05	0.301	0.15	0.043
	3.30– 6.30	1.48	0.494	1.13	0.376	0.14	0.045
	6.30– 9.30	1.28	0.426	0.61	0.203	0.11	0.038
	9.30– 8.00	3.64	0.347	1.50	0.143	0.38	0.036
	For 24 hours	9.02		5.15		0.94	
	Composite	8.86		5.16		0.93	
9 (bath)	8.00–12.40	2.08	0.445	2.27	0.486	0.23	0.049
	12.40– 4.20	1.79	0.488	1.49	0.407	0.16	0.043
	4.20– 7.35	1.77	0.545	2.17	0.670	0.14	0.044
	7.35–11.15	1.56	0.426	0.93	0.254	0.14	0.038
	11.15– 8.00	3.62	0.414	1.94	0.202	0.34	0.039
	For 24 hours	10.82		8.80		0.99	
	Composite	10.85		8.75		1.02	
10 (no bath)	8.00–12.00	1.72	0.430	1.79	0.447	0.18	0.044
	12.00– 3.00	1.45	0.484	1.42	0.474	0.12	0.040
	3.00– 6.30	1.65	0.470	1.00	0.314	0.14	0.040
	6.30–11.30	2.54	0.508	1.54	0.307	0.20	0.040
	11.30– 8.00	3.76	0.443	2.37	0.279	0.33	0.039
	For 24 hours	11.12		8.22		0.96	
	Composite	11.16		8.32		0.98	
11 (no bath)	8.00– 1.30	2.51	0.457	2.81	0.512	0.25	0.045
	1.30– 4.30	1.52	0.507	1.64	0.546	0.14	0.045
	4.30– 7.15	1.28	0.464	0.88	0.321	0.11	0.041
	7.15–11.15	2.06	0.515	1.20	0.299	0.16	0.041
	11.15– 8.00	3.67	0.420	2.32	0.265	0.36	0.041
	For 24 hours	11.04		8.85		1.02	
	Composite	10.91		8.79		0.99	

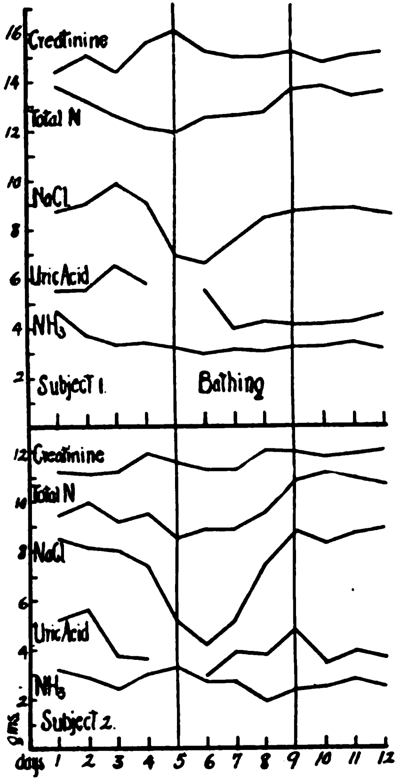

Fig. 1. Total nitrogen and sodium chloride are in grams, ammonia, uric acid and creatinine in tenths of grams, and all to the same base line except creatinine for which the zero is raised two units.

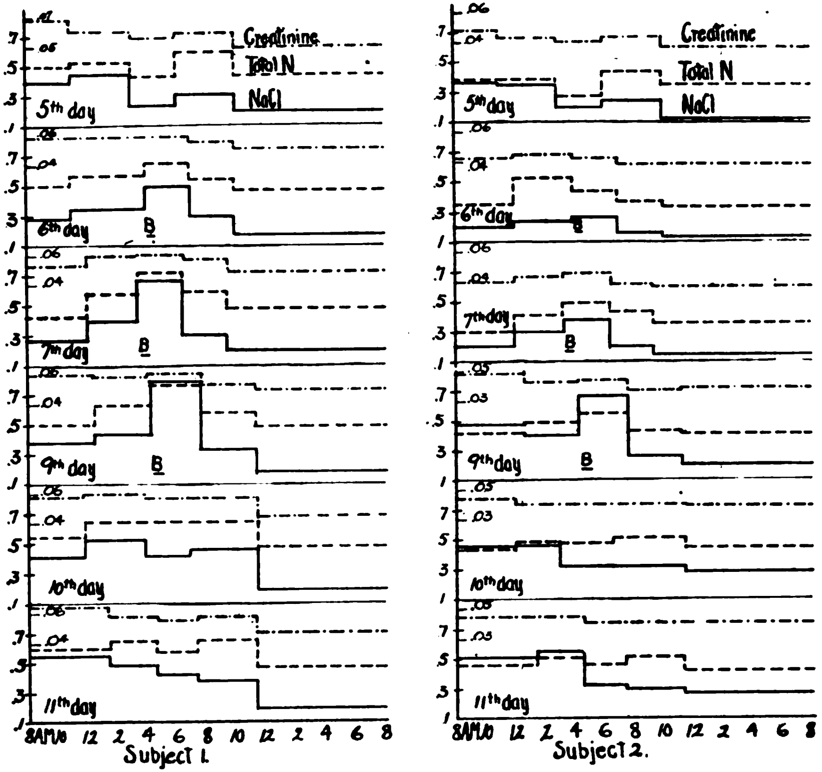

Fig. 2. Total nitrogen and sodium chloride in tenths of grams, creatinine in hundredths of grams.

ing output during the fore period, a progressively increasing output during the bathing period, which level is maintained during the final period.

The course of the chloride curves could be accounted for as follows: the uniform diet evidently contained less salt than the ordinary mixed diet which preceded it, so that during the fore period the effect of a lessened chloride intake gradually appears as a decreased salt excretion. With the beginning of bathing this relative retention of salt gradually gives way to increasing salt excretions which reach their maximum on the last day of bathing and remain at this level, or even slightly exceed it in Subject 2, in the final period. Nitrogen elimination follows practically the same course and this parallelism in nitrogen and chloride excretions was an unusual and unexplained finding in the previous experiment.

An examination of the data from the short period samples brings this out more clearly, and offers further evidence on the immediate effects of bathing. In figure 2 the third period on days 5, 10 and 11 (no bath) shows a marked difference from the same period on days 6, 7 and 9 (bath). When no bath was taken the rate of chloride and nitrogen excretion was lower during this period than during the preceding one, and usually lower than the rate in the succeeding period. On bathing days the rate of chloride and nitrogen excretion during this third period (at the beginning of which a bath was taken) was, in all cases but one, considerably higher than the rates observed both in the preceding and in the succeeding period. The progressively increasing daily output of nitrogen and chloride during the bathing period, as shown in figure 1, is largely caused, according to figure 2, by an increase in the rate of excretion immediately following the bath. The effect of the bath on metabolism is therefore primarily immediate though not entirely so; in both subjects the rate of excretion of nitrogen during the night period rises steadily reaching its maximum after the last bath, or on the day following, and the same statement may be made with reference to the chloride excretion of Subject 2.

An absorption of salt by the skin during the bath might explain the course of the chloride excretions as an attempt to restore chloride equilibrium, but the mechanism by which nitrogen excretion could be similarly involved is not clear. If the bathing brings about a mobilization of chlorides or their redistribution between the tissues and body fluids so as to cause their excretion, it would appear as if such a process were accompanied by an increased protein degradation or a flushing out of already existing decomposition products of protein. If on the other hand the metabolism of nitrogen is primarily affected by the bathing the increased chloride excretion is far above the amount that would be associated with a decomposition of body protein to the extent necessary to account for the higher urinary nitrogen. It is, in fact, entirely probable that body protein remains untouched by the response of the organism to the bath, at least as far as glycogen formation is concerned. In three experiments on the influence of cold (8° to 12°C.) baths on the glycogen content of man Lusk (2) found glycogen formation from protein in one case, an individual under normal weight. In those experiments the intestine was free from carbohydrates and while no values for body temperature are given, the general and intense shivering noted in his subjects never obtained in these brine baths and the alimentary tract was never free from carbohydrate at the time of the bath. This of course does not exclude the possibility of other influences outside of glycogen formation, which might modify protein metabolism. Whether these parallel findings on chloride and nitrogen excretion as modified by bathing depend upon the bath as such or upon the salt water must be determined by further experiments.

A chance deviation from the regular routine of the subjects is responsible for the suggestion that the increased nitrogen and chloride excretion by way of the kidneys might be merely the evidence of a lessened skin excretion. On the fifth day, which was warm, exercise in the form of tennis for an hour and a half was taken during the third collecting period, 3 to 6 p.m. Inasmuch as the exercise in the water on bathing days was not otherwise compensated for it was felt that this exercise, which was not violent, could be indulged in with impunity, but this was evidently not the case with reference to the skin excretion judging from the urinary nitrogen and chloride for the period. The hourly rate in this period was lower for this day than for the corresponding period on any of the non-bathing days in which period samples were taken.

Information upon the nitrogen and chloride content of the perspiration is somewhat fragmentary because of the variety of conditions under which observations have been made, but is sufficient to warrant the general statement that sweat usually contains from 0.05 per cent to 0.2 per cent of nitrogen and 0.5 per cent sodium chloride as a maximum concentration at the height of secretion rate (3, 4). Since Argutinsky (5) found 0.7 grams of nitrogen in the perspiration collected during a seven-hour walk on a hot day there would have been about 0.15 gram nitrogen and 0.38 gram sodium chloride[2] in the perspiration excreted during an hour and a half of tennis, values which will explain the diminished amounts of urinary nitrogen and sodium chloride in the third period of the fifth day. That the cooling of the skin by bathing should prevent the elimination of perspiration sufficient to account for the higher urinary concentration in nitrogen and chlorides in the third period of the bathing days is scarcely a justifiable conclusion, and this question must be subjected to further investigation. The fact that body temperature is not markedly affected by the bath (1) and that the increasing chloride and nitrogen excretion during the bathing period continues at approximately its highest level during the final period indicates that other factors besides the urine-sweat partition are operating.

The rate of creatinine excretion as shown by the data obtained from the short period samples does not indicate that the bathing had any influence upon it; the data rather emphasize the constancy of the excretion rate of creatinine.

Several blood pressure measurements before, during and after the bath were made by means of a Tycos sphygmomanometer and auscultation over the radial artery. The values (systolic) obtained for Subject 1 were uniformly 110, for Subject 2, 105, and the expected rise in blood pressure during the bath was not shown by this somewhat approximate method.

SUMMARY

Two subjects were maintained on a uniform diet for twelve days. A bathing period of four days followed a fore period of five days, with a three day final period. The urine was analyzed in 24-hour periods on all of the days and in short periods (3 to 4 hours) on three bathing and on three non-bathing days. In agreement with former results the progress of the bathing period was attended by increased nitrogen and salt excretion, which, in this case, however, persisted through the final period. The three-hour period during and immediately following the bath, showed a considerable increase (15 to 50 per cent) in nitrogen and salt excretion as compared with the same period on days when no bath was taken, indicating that the bath had an immediate influence as well as a prolonged effect. A constant and uniform parallelism between nitrogen and chloride variations, noted also in earlier work, is not understood. Decreased perspiration through the cooling of the skin by the bath can account in only small measure for the greater salt and nitrogen excretion in the urine following the bath.

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[2] This calculation for sodium chloride is made on the assumption that the nitrogen excretion of 0.7 gram was 0.2 per cent of the total perspiration, which would therefore have been 350 cc. in the seven hours, or 75 cc. in an hour and a half, 0.5 per cent of which would be 0.38 gram of sodium chloride. This calculation is of course based on unjustified assumptions as to the constant rate of excretion of perspiration by different persons and its constant composition.

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RELATION BETWEEN THE AMOUNT OF CATALASE IN THE DIFFERENT MUSCLES OF THE BODY AND THE AMOUNT OF WORK DONE BY THESE MUSCLES

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Oxidase in plants and in animals has been the subject of much investigation. For a review of the literature on the subject see Oppenheimer, "Die Fermente und ihre Wirkungen," 1913 and Kastle, "Hygienic Laboratory Bulletin No. 59, 1909." The presence of catalase in the tissues has been demonstrated by many observers (1) employing a variety of reactions by which colored oxidation products are formed within the tissues. These observers (2) have shown that the capacity of the tissues to form these colored products has a direct relation to their capability of freeing oxygen from hydrogen peroxide, of bluing tincture of guaiac and of oxidizing salicylic aldehyde and benzyl alcohol to their respective acids. They found that there are definite and constant differences in the oxidative properties of the different tissues. This property was most marked in the spleen, liver and kidney and least marked in muscular and nervous tissue.

Although a great amount of work has been done in this field, at the same time, so far as I know, no one has attempted to correllate the amount of substance in a muscle that will liberate oxygen from hydrogen peroxide with the amount of external physical work done by the muscle. This substance will be referred to hereafter in this paper as catalase. The object of this investigation was to determine if there was more catalase in a muscle normally doing a relatively large amount of work than in one normally doing a relatively small amount of work, and if by increasing or decreasing the amount of work the amount of catalase would be correspondingly increased or decreased. The muscles of which the catalase content was to be determined were removed from the animals after the blood vessels had been washed free of blood by the use of large quantities of 0.9 per cent sodium chloride and hashed in a hashing machine. The amount of oxygen liberated in ten minutes from 30 cc. of hydrogen preoxide by one gram of the hashed muscle was taken as a measure of the amount of catalase in the muscle. The solution of hydrogen peroxide used was prepared by diluting 15 cc. of commercial hydrogen peroxide to 30 cc. with distilled water.

Method. The apparatus used in this investigation in determining the amount of catalase is shown in figure 1; *a* and *a'* are two inverted burettes with their mouths beneath the water in a glass vessel *f;* *e* and *e'* rubber tubes connecting bottle *d* with the burettes; *b* and *b'* stopcocks and *c* a rubber stopper. The burettes were filled with water by sucking on *a* and *a'* and the stopcocks of the burettes closed. One gram of the hashed muscle was placed in a small crucible which was introduced into bottle *d,* rubber stopper *c* having been removed. Thirty cubic centimeters of the diluted hydrogen peroxide were introduced into the bottle care being taken that none of the hydrogen peroxide came in contact with the muscle at this time. Rubber stopper *c* was placed firmly in the mouth of the bottle while stopcocks *b* and *b'* were open. By turning the stopcocks of the burettes thus admitting air, the meniscus of the water in each burette was brought to the zero marking. Stopcock *b'* was then closed while *b* remained open. Bottle *d* was tilted and the contained crucible turned over thus mixing the hydrogen peroxide and the hashed muscle. The bottle was gently shaken for 10 minutes while the oxygen gas was being liberated and conducted by the rubber tube into the burette where it displaced the water. At the end of the ten minutes stopcock *b* was closed and the amount of gas read off directly from the burette. After making the necessary calculations for reducing the volume of the gas to standard

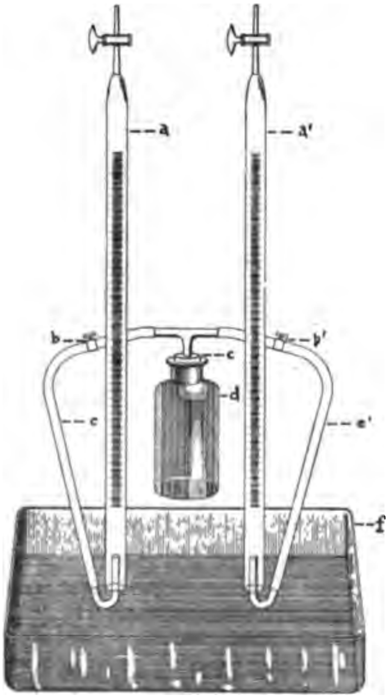

FIG. 1.

atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in one gram of the muscles. The second burette *a'* was used when more than 100 cc. of oxygen was given off, each burette being of 100 cc. capacity. The temperature of the gas in the apparatus was kept fairly constant, 20°C., during the determinations.

In the manner described above determinations were made of the amounts of oxygen liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in one gram of the hashed muscle of the heart and of the legs (biceps, gastrocnemius, semi-membranous, semi-tendinous) of dogs, rabbits, pigeons, rats, turtles and frogs; of the back (latissimus dorsi, trapezius serratus posterior inferior) of rabbits and rats; of the head (temporalis) of dogs and of the breast (pectoralis) of pigeons. These muscles will be referred to hereafter in this paper as heart, leg, back, head and breast muscles respectively.

TABLE 1

After heart, leg, and head are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of dogs

DOG	1	2	3	4	AVERAGE
Heart	70	75	72	67	71
Leg	55	54	50	56	54
Head	32	39	33	31	35

Dogs. The four dogs used in the following experiments were tramp dogs obtained through the local dog catcher. The heart, leg and head muscles of these animals were washed free from blood and were then removed and ground up in a hashing machine. The amount of oxygen liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in one gram of each of these groups of muscles was determined according to the method described. The results of these determinations are given in table 1. It may be seen that the average amounts of oxygen liberated by the heart muscle of the four dogs is 71 cc.; that by the leg muscles is 54 cc.; and that by the head muscles is 35 cc. It is assumed that the amount of catalase in these different groups of muscles is proportional to the amount of oxygen liberated, hence the amount of catalase in the leg muscles is 76 per cent of that in the heart and that in the head muscles 49 per cent. It would seem to be a fair assumption that of these muscles of the dog the heart under normal conditions does the largest amount of work, the head muscles the least, and the leg muscles an intermediate amount, hence the amount of catalase in these muscles is proportional to the amount of work done.

Rabbits. These were born and raised in captivity. The heart, leg and back muscles were removed from these animals after the blood vessels had been washed free of blood with 0.9 per cent sodium chloride. They were then ground up in a hashing machine. The amounts of oxygen liberated in ten minutes by one gram of the different groups of muscles from 30 cc. of hydrogen peroxide is given after heart, leg and back in table 2. It may be seen that the average amount of oxygen liberated by the heart muscle of the four rabbits is 114 cc.; that by the leg muscles is 49 cc.; and that by the back muscles is 35 cc. Hence the amount of catalase in the leg muscles is 43 per cent of that in the heart and that in the back muscles is 30 per cent. As in the case of the dogs the amount of catalase in the adult rabbits is greatest in the heart muscle, and intermediate in amount in the leg muscles.

TABLE 2

After heart, leg and back are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of rabbits

RABBIT	1	2	3	4	AVERAGE
Heart	110	100	115	130	114
Leg	52	48	50	46	49
Back	40	36	38	31	35

Pigeons. The pigeons used in these experiments were ordinary male and female domestic pigeons accustomed to the range of the town. After the blood vessels had been washed free of blood with 0.9 per cent sodium chloride the heart, breast and leg muscles were removed and ground up. The catalase content of these muscles was determined as in the preceding experiments. The results of the determinations for four active male pigeons and four inactive sitting females are given in table 3. It may be seen that the heart muscle contained the largest amount of catalase, the breast muscles an intermediate amount, while the leg muscles contained the smallest amount. The high catalase content of the breast muscles is accounted for by the fact that normally these muscles do much work in the act of flying. The catalase content of the heart and breast muscles of the active male pigeons is somewhat higher than that of the inactive sitting females. This may be accounted for by the fact that the muscles of the active males had been accustomed to do more work than those of the inactive sitting females.

From the preceding experiments it would seem that catalase is greatest in amount in muscles doing the greatest amount of work, least in amount in those doing least work and intermediate in amount in those doing an intermediate amount of work. It has been recognized for a long time that oxidation is more intense in muscles doing a great amount of work than in those doing a small amount of work and since catalase is greatest in amount in muscles doing the largest amount of work and least in amount in muscles doing least work it follows that the amount of catalase varies with the intensity of the oxidation in the muscles.

TABLE 3

After heart, breast and leg are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of active male pigeons, of inactive sitting females

Active males

PIGEON	1	2	3	4	AVERAGE
Heart	111	102	120	115	112
Breast	90	92	96	98	94
Leg	59	63	66	64	63

Inactive sitting females

PIGEON	1	2	3	4	AVERAGE
Heart	92	98	109	104	101
Breast	87	86	89	93	89
Leg	52	60	58	62	58

Turtles and frogs. In table 4 under turtle and frogs are given the amounts of oxygen liberated by the heart and leg muscles of these animals. It may be seen that the average amount of oxygen liberated by the heart muscle of four turtles is 31 cc. and of the leg muscles 19 cc.; that of the leg muscles of the frogs from Chicago 18 cc., and that of the leg muscles of the frogs from New Orleans 22 cc. It may be recalled that the leg muscles of the dog, rabbit and active male pigeons liberated 54 cc., 49 cc., and 63 cc. of oxygen respectively and that the heart muscles liberated 71 cc., 114 cc. and 112 cc. If these amounts of oxygen be compared with those liberated by the corresponding muscles of the turtle and frog it will be seen that they are much greater. It has been recognized for many years that oxidation in a warm blooded animal such as a dog is much more intense than in a cold blooded animal such as the frog. From these experiments it would appear that catalase is greater in amount in the muscles of warm blooded animals in which oxidation is more intense than in the muscles of cold blooded animals in which oxidation is less intense.

TABLE 4

After heart and leg are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of turtles and frogs

Turtles from Chicago

TURTLE	1	2	3	4	AVERAGE
Heart	27	32	29	35	31
Leg	16	18	24	19	19

Small frogs from Chicago

FROG	1	2	3	4	AVERAGE
Leg	22	15	18	17	18

Jumbo bullfrogs from New Orleans

BULLFROG	1	2	3	4	AVERAGE
Leg	20	19	27	24	22

The following experiments were carried out to determine the effect of confinement on the catalase content of the heart, leg and breast muscles of male pigeons similar to those used in the preceding experiments. Nine pigeons were placed in a small cage so that there was no opportunity for the use of their breast muscles in flight. The catalase content of the heart, leg and breast muscles of three of these pigeons was determined before the period of confinement was begun. Similarly determinations were made at the end of the fifth, the tenth and the thirtieth days of confinement. The average amounts of oxygen liberated by the different groups of muscles of these pigeons are given after heart, leg and breast in table 5.

It will be observed that there had been little reduction in the catalase content of the heart and leg muscles of the confined pigeons. The catalase content of the breast muscles, however, had fallen during the period of confinement as is indicated by the decrease in the amount of oxygen liberated from 89 cc. to 75 cc. after five days confinement, from 89 cc. to 59 cc. after ten days confinement and from 89 cc. to 58 cc. after thirty days confinement. The fact that the catalase content of the leg and heart muscles had decreased very little during confinement may be accounted for by the fact that these particular muscles were doing about the same amount of work during confinement as when the pigeons were at liberty. The physical work of the breast muscles of the pigeons, however, being reduced practically to zero during confinement was accompanied by a decrease in the catalase content of the muscle of approximately 35 per cent as is indicated by the decrease from 89 cc. to 58 cc. in the amount of oxygen liberated.

TABLE 5

After heart, breast and leg are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of pigeons confined for 5, 10, and 30 days

PIGEON	0 DAYS CONFINEMENT	5 DAYS CONFINEMENT	10 DAYS CONFINEMENT	30 DAYS CONFINEMENT
Heart	109	104	103	101
Breast	89	75	59	58
Leg	57	56	57	54

The experiments now to be described were carried out to determine the effect of exercise on the catalase content of the heart, leg and back muscles of rats. Twelve ordinary barn rats, caught in traps, were placed in a small cage so that there was little opportunity for exercise. The catalase content of the heart, leg and back muscles of four of these rats was determined soon after they were caught. The average amount of oxygen liberated by one gram of the hashed muscles of these rats is given in table 6 after "Rats (unconfined)." The heart muscle liberated 109 cc. the leg muscles 38 cc., the back muscles 29 cc. The remaining rats were kept in a small cage for one month. At the end of this time the catalase content of the heart, leg and back muscles of four of these rats was determined. In the table after "Rats (confined)" is given the amounts of oxygen liberated by the heart, leg and back muscles of these rats. It may be seen that the catalase content of all of these muscles had decreased as is indicated by a decrease in the amount of oxygen liberated.

If the effect of confinement on the catalase content of the muscle of the pigeons and of the rats be compared it will be found that but little change had been produced in the catalase content of the hearts of these animals by confinement; that the catalase content of the muscles of the legs of the pigeons was not changed while it was decreased in the muscles of the legs of the rats. This difference in effect is accounted for by the fact that the physical work of the muscles of the legs of the pigeons was about the same during confinement as when the pigeons were at liberty while the physical work of the muscles of the legs of the rats was reduced during confinement with a corresponding reduction in the catalase content of the muscles.

TABLE 6

After heart, leg and back are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalse in 1 gram of the respective muscles of active unconfined rats, inactive confined rats and rats exercised

Rats unconfined

RAT	1	2	3	4	AVERAGE
Heart	110	100	120	106	109
Leg	36	34	39	42	38
Back	25	28	32	30	29
Rats confined					
Heart	96	93	99	100	97
Leg	30	32	28	34	31
Back	26	20	30	24	25
Rats exercised					
Heart	104	96	101	108	102
Leg	38	40	46	44	42
Back	31	34	28	29	30

The four remaining rats that had been confined in a small cage for a month were given exercise in the following manner. An ordinary circular cheese box 35 cm. in diameter was attached to a motor reducing gear. A piece of circular wire netting with door cut in it was used as a top for the box. The box was adjusted so that it could be rotated with its vertical axis in an horizontal direction. The rats were introduced into the box and it was rotated at a speed of fourteen revolutions per minute six hours each day, three hours in the morning and three hours in the afternoon for six successive days. This speed of rotation kept the rats walking in a comfortable manner. At the end of the six days the catalase content of the heart, leg and back muscles of the animals was determined. In table 6 after "Rats (exercised)" is given the amount of oxygen liberated by the respective muscles. It may be seen that the average amount of oxygen liberated by the heart muscle was 102 cc., that by the leg muscles 42 cc.; that by the back muscles 30 cc. By comparing these amounts of oxygen with those liberated by the corresponding muscles of "Rats (confined)" it may be seen that exercise had increased the amount of catalase in the leg and back muscles as is indicated by the increase in the oxygen liberated while little change had been produced in the catalase content of the heart muscle.

It is recognized that oxidation can be increased or decreased in a muscle by increasing or decreasing the amount of work done by the muscle. It may be seen in the preceding experiments with pigeons and rats that when the work of the muscles was increased with the resulting increase in oxidation the amount of catalase was increased and vice versa.

CONCLUSIONS

1. The amount of catalase in the different muscles of the body varies with the amount of work done by these muscles; those doing the greatest amount of work contain most catalase, while the muscles doing the least work contain least catalase.
2. By increasing or decreasing the external physical work of a muscle the amount of catalase is correspondingly increased or decreased. If the external physical work of a muscle such as the pectoralis of pigeons is reduced practically to zero the amount of catalase is reduced by approximately 38 per cent.
3. Catalase is greater in amount in the muscles of warm blooded animals in which oxidation is more intense than in corresponding muscles of cold blooded animals in which oxidation is less intense.

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A FURTHER STUDY OF THE BLOOD GASES DURING HIBERNATION IN THE WOODCHUCK (MARMOTA MONAX) THE RESPIRATORY CAPACITY OF THE BLOOD

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INTRODUCTION

In a former paper (1) it was shown that during hibernation of the woodchuck there is an increase of CO_2 in the blood from about 70 volumetric per cent to as high as 100 volumetric per cent in the case of the venous blood, and from about 68 volumetric per cent to as high as 87 volumetric per cent in the arterial blood. This confirmed the observations of Dubois on the European marmot. In discussing the results it was recognized that this difference in the blood of the two states might have been exaggerated to some extent because of the use of ether in immobilizing the active animal, while no anaesthetic was used on the dormant ones. It was concluded from a single case in which chloretone was used that the probable error was slight. In order to make sure of this point, it was finally decided to make a more extensive series of observations along a similar line except that some other means be used to immobilize the active animal.

A saturated aqueous solution of chloretone was found to be very slow in its action and to involve the injection of too large a quantity of water to be absorbed and thus tend to dilute the blood. An alcoholic solution was tried and found to be more satisfactory, especially when aided by cocaine as a local anaesthetic, if given in a large enough dose. Due, however, to the death of about fifteen woodchucks from some unknown cause, the number of animals available for this experiment was unintentionally limited.

It was also mentioned in the paper referred to above that DuBois (2) concluded that the respiratory capacity of the blood was greater during hibernation because of an increase in the concentration of the blood. He found that defibrinated blood obtained from a hibernating marmot with a rectal temperature of 8.6°C. when treated for one hour at 12°C. with a current of air and CO_2, contained 124.7 volumetric per cent CO_2, 18.6 volumetric per cent 0_2 and 2.9 volumetric per cent N. The same treatment in summer in the case of the blood of an active animal with a rectal temperature of 34.2°C. yielded only 103.55 volumetric per cent CO , 13.06 volumetric per cent 0_2 and 2.6 volumetric per cent N. Somewhat similar figures to these latter were obtained from an animal which while dormant in the winter had been bled and allowed to slowly warm up at the rate of 2.6°C. in four hours, and which was bled a second time when the marmot had a rectal temperature of 35.4°C. The blood obtained at this second bleeding and treated in the same way had less oxygen in it than the blood of the active animal, otherwise it was very similar. This indicated that the cause of the increased respiratory capacity of the blood during winter-sleep was due to its increased concentration. To strengthen this belief was the fact that the specific gravity of the non-defibrinated arterial blood of a torpid marmot was 1.071 while that of the defibrinated was 1.0654 as compared with 1.046 for non-defibrinated and 1.0525 for defibrinated arterial blood of an active animal.

From an examination of the specific gravity of the blood of a few woodchucks, some of which were killed during the active state and others during hibernation, it appeared that no such striking increase in the specific gravity of the blood occurred in these animals. It was, therefore, interesting to know if the respiratory capacity of the blood similarly showed no increase, granting that the conclusions of Dubois were correct. A series of experiments were carried out to test this point and to see the relationship of the respiratory capacity of the blood to its actual oxygen and carbon-dioxide content in both the active and the dormant state.

METHOD

One hour before the operation of exposing the blood vessels commenced, the animal (whether torpid or active) was given per rectum for each kilogram of body weight 2 cc. of 50 per cent alcohol containing 0.3 gram of chloretone, which is twice the dose prescribed for the rabbit. This gradually immobilized the active animal without excitation, and did not disturb the hibernating ones. With the additional use of a little cocaine as a local anaesthetic, the operation of exposing the vessels could be done without exciting the animal.

As a rule the hibernating animals of this series were not as dormant as is the general rule. Since the carotid artery, which is much more inaccessible especially in the woodchuck on account of such a short neck, was used instead of the femoral artery, the chloretone treatment during winter-sleep served to lessen the possibilities of disturbing the animal during the operation. Thus as many factors as practicable were kept constant throughout the whole series.

The apparatus used and the further procedure were essentially the same as that described in the previous paper, except that only three manometers were used on each sample of blood. The apparatus being in better working order and the manipulations having become more accurate as a result of increased experience, a larger number of duplicate analyses were not necessary. The venous blood was collected by means of an ordinary cannula from a large deep branch of the jugular, thus doing away with the necessity of the T tube used as a cannula in the previous series.

About 2 cc. of blood of each kind (venous and arterial) was drawn into two sampling pipettes. Then nearly 6 cc. of arterial blood was collected in a specific gravity bottle, and finally 20 cc. of arterial blood was collected and defibrinated in a small flask containing glass beads. This defibrinated blood was filtered through absorbent cotton and divided into two equal portions. Through one portion was passed, for one hour at a temperature of 20°C., a steady stream of oxygen which had just been washed in water. The other portion was treated similarly, for one hour also at 20°C., with a stream of carbon dioxide which had just been passed through a solution of sodium bicarbonate. The blood being saturated with these gases was kept in closed vessels during the process and the rate of the flow of gas was kept as nearly equal as practicable throughout the whole series. Whatever evaporation or dilution that might result during this process ought to be fairly uniform.

While the defibrinated blood was being saturated with gases, the first samples of blood taken were analysed for oxygen. Then the two samples of defibrinated blood, which had by this time become saturated, were analysed for oxygen. Finally the analysis of all the samples for carbon dioxide was carried out together. If care was taken in handling the defibrinated blood which had been saturated with carbon dioxide so as to expose it to the air only momentarily and then to quickly take a sample from the bottom and unexposed portion, such blood contained no oxygen, and similarly the blood saturated with oxygen contained no carbon dioxide. These items, consequently, do not appear in the tables of results which follow.

RESULTS

The results are tabulated in the three accompanying tables. The data as seen in the first two tables below confirm in general those obtained in the earlier series, and show that the amount of CO_2 normally present in the active woodchuck is comparatively high, being nearly

TABLE 1

Blood gases before hibernation

ANIMAL (SERIES III)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL		
		A = Arterial V = Venous D = Difference	O_2	CO_2	Rectal temperature	Reduced body weight	Remarks
					deg. C.	*grams*	
32	October 14, 1915	A	19.91	64.85	37.3	3,504	Active
		V	16.29	75.16			
		D	3.62	10.31			
33	October 19, 1915	A	19.40	77.55	36.7	1,830	Active
		V	16.00	86.30			
		D	3.40	8.75			
34	October 23, 1915	A	19.41	78.62	35.6	1,925	Active
		V	13.26	85.70			
		D	6.15	7.08			
35	October 26, 1915	A	23.21	66.62	37.1	2,400	Active
		V	16.64	74.23			
		D	6.57	7.61			
36	October 30, 1915	A	20.84	62.95	34.7	1,900	Active
		V	10.19	77.51			
		D	10.65	14.56			
38	November 13, 1915	A	19.47	70.61	33.9	2,688	Active
		V	16.02	75.99			
		D	3.45	5.38			
41	December 4, 1915	A	16.80	67.00	34.0	1,258	Active
		V	10.80	72.51			
		D	6.00	5.51			
Average...............		A	19.86	69.75	35.2	2,215	
		V	14.17	78.20			
		D	5.69	8.46			
Average of active animals of the earlier series where ether was used		A	19.07	67.86	31.7		
		V	13.04	70.08			
		D	6.03	2.22			

TABLE 2

Blood gases during hibernation

ANIMAL (SERIES III)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL		
		A = Arterial V = Venous D = Difference	O_2	CO_2	Rectal temperature	Reduced body weight	Remarks
					deg. C.	*grams*	
44	December 23 1915	A	22.69	68.87	13	3,051	Dormant. Had a rectal temperature of 9°C. five days previously.
		V	7.18	90.34			
		D	15.51	21.47			
45	December 28 1915	A	20.50	79.13	10	2,046	Dormant
		V	15.33	94.01			
		D	5.17	14.88			
46	February 1 1916	A	17.44	60.74	9	1,780	Dormant. All but one other animal in these burrows were awake and active
		V	12.07	68.96			
		D	5.37	8.22			
47	February 3 1916	A	19.36	65.52	6	1,580	Dormant. Also asleep two days previously. Other animals in the same burrow awake with rect. T. 35°C.
		V	9.68	81.63			
		D	9.68	16.11			
52	March 4 1916	A	20.51	84.97	10	1,203	Very dormant
		V	19.70	99.75			
		D	.81	14.78			
53	March 6 1916	A	25.38	87.99	12	1,849	Very dormant
		V	13.30	94.52			
		D	12.08	6.53			
Average..........		A	20.98	74.51	8.6	1,918	
		V	12.88	88.20			
		D	8.10	13.69			

70 volumetric per cent as an average of seven animals. This is slightly less than 2 volumetric per cent higher than was obtained when ether was used—the average of the six determinations made with ether as the anaesthesia being placed at the end of table 1 for ready comparison.

All other figures also compare well, the variations being as close as could be expected from the limited number of animals involved, except the lower percentage of CO_2 in the venous blood of the ether series.

There is on an average only a difference of 2.22 volumetric per cent in the CO_2 in the venous and in the arterial blood where ether was used, as compared with 8.46 volumetric per cent difference in the chloretone series. This error, while somewhat great when taken alone, does not seriously modify the general difference observed between the gases in the blood of the two states.

By comparing tables 1 and 2 it will be seen that the CO_2 content of the blood is much higher during hibernation, reaching nearly 100 volumetric per cent in animal 52. The average per cent of CO_2 in the blood of this series of six hibernating animals is probably not representative because of animals 46 and 47. During the latter part of January and early part of February all our woodchucks, except these two (46 and 47) were awake and more or less active, some of them having a rectal temperature of 35°C. Since it was desirable to get analyses during the middle part of the hibernating period, the only two animals that were dormant were utilized. These two woodchucks were freely accessible to the active ones since the burrows communicated, and had undoubtedly been disturbed. It has been repeatedly shown that when hibernating animals are disturbed and awakening commences, there is a great increase in the elimination of CO_2 (3). In general the duration and depth of torpor obtained during this last winter was unmistakably much less than that obtained during the preceding season. The unusually mild weather which existed for a long period in January naturally suggests itself as having had something to do with this unusually long period of awakening, although the relation of cold to winter-sleep is not agreed upon.

The fact that the two woodchucks under consideration were asleep while there was practically no excess of CO_2 in the blood, has a bearing upon the theory of Dubois, namely that the accumulation of an excessive amount of CO_2 in the blood and tissues of hibernating animals is an essential condition in maintaining dormancy during winter sleep. Here are apparently two cases of torpor with practically no excess of CO_2, unless these particular animals in the active state naturally had much less CO_2 in the blood than has been the case in any woodchuck so far investigated. There is, of course, always this possibility. Then again there is the possibility that the excess of CO_2 in the system may only be a result of altered ventilation and a decrease in the irritability of the respiratory center. The air in the burrows must become heavily charged with CO_2 because of the comparatively small amount of air confined within when the snow covers the ground. If the respiratory center during this period is less sensitive to the increased H ion concentration which would follow the higher CO_2 pressure in the alveolar air, CO_2 would naturally accumulate in the blood and tissues.

A further comparison of tables 1 and 2 shows that there is distinctly a greater difference between the amount of CO_2 in the venous and in the arterial blood of the hibernating animal. Similarly, there is a greater difference between the amount of 0_2 in the arterial and in the venous blood during winter sleep.

In the previous paper, it was stated that a number of observers (Valentin, Quincke, Claude Bernard, Marès, Allen Cleghorn) had noted that the blood of hibernating animals was unusually bright in the veins, leading some of them to conclude that all the blood was arterial during winter-sleep. In animal 52 there is nearly as much 0_2 in the venous blood as in the arterial. No difference in color could be detected after being collected in the sampling pipettes. This is the only case where in an undisturbed animal such a condition was observed. It may be noted, however, that the maximum percentage of 0_2 recorded in any case where there was no evidence of overventilation in either series was found during hibernation. In the ether series it amounted to 25.76 volumetric per cent in the arterial blood of a torpid animal (rectal temperature 6°C., no anaesthesia) examined December 19, 1914. In the chloretone series it occured also in the arterial blood of a dormant animal (rectal temperature 12°C., chloretone) examined March 6, 1916, and amounted to 25.38 volumetric per cent.

The results of saturating the defibrinated arterial blood with CO_2 and 0_2 are found in the table below in connection with other related data.

The table shows that during hibernation there is in the woodchuck no difference in the 0_2 absorbing capacity of the blood, but a distinct decrease in the power to absorb CO_2, notwithstanding the fact that the average specific gravity of the blood of these particular dormant animals is slightly greater than that of the active ones. The average weight of the active animals is but slightly greater than that of the torpid ones, hence this difference is not due to having used smaller animals during the winter, so that the withdrawal of the same amount of blood would represent in the smaller hibernating animal a relatively greater hemorrhage and cause a greater dilution of the blood by the influx of lymph as the blood pressure falls. It may also be mentioned here that during winter-sleep the blood volume probably decreases slightly less rapidly than does the body weight as a whole, at least the percentage of blood does not decrease below that found just before hibernation. The data supporting this statement will be published later. Hence the blood volume of the hibernating animals used here is undoubtedly fully equal to that of the active ones.

TABLE 3

Respiratory capacity of the blood

ANIMAL (SERIES III)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF DEFIBRINATED BLOOD REDUCED TO 0°C AND 750 MM. PRESSURE		SPECIFIC GRAVITY OF BLOOD	CONDITION OF ANIMAL		
		O_2	CO_2		Rect. temp.	Reduced body weight	Remarks
			Before hibernation				
					deg C.	*grams*	
33	October 19, 1915	20.88	222.48	1.0573	36.7	1830	Active
34	October 23, 1915	24.42	247.34	1.0579	35.6	1925	Active
35	October 26, 1915	24.03	235.46	1.0646	37.1	2400	Active
36	October 30, 1915	21.16	212.69	1.0577	34.7	1900	Active
38	November 13, 1915	24.88	205.12	1.0596	33.9	2688	Active
41	December 4, 1915	16.14	216.40	1.0562	34.0	1258	Active
Average		21.92	223.25	1.0589	35.3	2000	
			During hibernation				
44	December 23, 1915	22.39	215.79	1.0620	13	3051	Dormant
45	December 28, 1915	24.86	197.81	1.0647	10	2046	Dormant
46	February 1, 1916	19.13	189.21	1.0571	9	1780	Dormant
47	February 3, 1916	21.18	203.79	1.0631	6	1580	Dormant
52	March 4, 1916	20.18	217.26	1.0565	10	1203	Dormant
53	March 6, 1916	23.92	231.88	1.0628	12	1849	Dormant
Average		21.94	209.29	1.0610	10	1918	

The results show also that the blood of the woodchuck has an extraordinary capacity for absorbing CO_2. In the six active animals it ranges from 205.12 volumetric per cent to 247.35 volumetric per cent with an average of 223.25 volumetric per cent. In the six hibernating animals the limits are 189.21 volumetric per cent and 231.88 with an average of only 209.29 volumetric per cent. Dubois maintains that the respiratory capacity of the blood increases during winter-sleep in the case of the European marmot. His investigation was, however, limited to three animals and in these the variation in the specific gravity of the blood was far greater than has ever been found in the woodchuck in this laboratory, where up to the present time the specific gravity of the blood of about 50 woodchucks has been determined.

In view of the fact that during inanition, especially of man and other animals whose food is partly or entirely of a vegetable character, there is a decrease in the alkalinity of the blood, i.e., a tendency towards acidosis as deducted from the analysis of the urine and alveolar air (4), one would expect the amount of CO_2 capable of being absorbed by the blood to be distinctly less during hibernation, of herbivorous animals at least. The woodchuck is strictly herbivorous, but during winter-sleep it lives entirely on its own tissues and so may be considered a carnivor. Of the tissues consumed during hibernation a very large fraction is adipose. The respiratory quotient is, therefore, small during lethargy. It has been shown by several authors (5) that the respiratory quotient may fall as low as 0.5, thus indicating not only that the food is fat and proteins, but also that there is a retention of oxygen, undoubtedly as CO_2 and probably also in the form of some incompletely oxidized products. A fat-rich diet is now looked upon as tending to produce acidosis because of incomplete oxidation of the fats and consequently the formation of acetone bodies (acetone, aceto-acetic, B-oxybutyric acid). (6)

That the withdrawal of the alkalies normally supplied in a vegetable diet and utilization of purely animal matter (the tissues of the body) as occur with the onset of hibernation, does increase the acid radicals (other than CO_2) relative to the basic radicals in some hibernating animals is in general supported by the meagre facts available on the reaction of the urine of such animals. Thus Dubois (7) found invariably that the urine of the marmot during the summer when fed on its normal vegetable diet was alkaline, while that of the dormant animal was acid. From Polimanti's (8) review of the literature there is some disagreement, however. Horvath reports that the spermophile voids a large quantity of alkaline urine when first it wakes up from winter sleep. Albini found that the urine discharged by the marmot when it wakes is neutral, while Quincke's examination of the urine of a hibernating marmot indicated a slightly acid reaction, with some albumin and skatole, but no indican. I. Monti explained the difference in the reaction of the urine upon a difference in the amount of gastric juice in the stomach. Polimanti's own observations (p. 351) are in agreement with those of Dubois on the alkaline character of the marmot's urine in the active state while being fed its usual vegetable diet. He agrees also with Dubois and Quincke on the acid reaction of the urine during hibernation. While nothing has been done on the urine of the woodchuck, there is every reason to believe that the conditions are the same as in the European marmot. The figures given by Noe (9) on the acidity of the urine of the hedgehog (an insectivore), excluding the single case reported in June, show a low acidity in January and an increase in acidity with the progress of winter, reaching a maximum in March (corresponding probably to the end of hibernation) after which there is a decrease again.

It is well known that weak acids readily drive out the CO_2 from the blood. It was, for example, shown by Walter (10) many years ago that the introduction of hydrochloric acid into the stomach of the rabbit may reduce the CO_2 in the blood to only 2 or 3 volumetric per cent. It is but to be expected that during hibernation of the woodchuck there will be sufficient acidosis to distinctly decrease the power of the blood to absorb CO_2. The fact that the blood actually contains more CO_2 during the dormant period would indicate that the respiratory center has become much less sensitive to the H ion concentration of the blood and that the CO_2 pressure in the alveoli of the animal must also be very high in order to maintain such a large amount of CO_2 in the blood in the presence of a probable reduction in its alkalinity.

It is strange that the arterial blood does not contain less oxygen during hibernation than during the active state, if such an increase in the H ion concentration actually occurs. Following the discovery by Bohr, Hasselbalch and Krogh (11), that an increase in the amount of CO_2 in the blood increases the relative amount of reduced hemoglobin, it was found that other acids also decreased the effective concentration of oxygen and that the effect is due to the H ion concentration (12). The results above show, however, that there is, if any thing, more oxygen in the arterial blood during hibernation than during the active state.

SUMMARY

1. A determination of the blood gases after treating woodchucks with chloretone, indicates that the use of ether in the previous series of experiments on blood gases during hibernation decreased somewhat the amount of CO_2 in the venous blood of the active animal.

2. All other figures in this series agree with those obtained in the first series as closely as could be expected, and hence show the same general

condition—that there is a relatively high CO_2 content in the blood of the woodchuck even when awake and active, that there is a marked increase in the amount of CO_2 in the blood during hibernation and especially during the latter part of this torpid state, and that the difference in the amount of gases in the venous and in the arterial blood is greater during winter-sleep than during the active state.

3. A single case was found in which during hibernation the venous blood could not be distinguished from the arterial by its general appearance. This venous blood upon analysis contained only 0.81 volumetric per cent less oxygen than did the arterial. This condition, which has been reported as being the general rule in the dormant state of hibernating animals by a number of observers, is only rarely found in the woodchuck.

4. The oxygen absorbing power of the blood is not changed during hibernation.

5. The absorbing power of the blood for CO_2 decreases during hibernation This may be ascribed to a probable decrease in the alkalinity of the blood.

Again the encouragement and direction received from Dr. Sutherland Simpson during the progress of this investigation is acknowledged, as is also the assistance rendered by Gertrude B. Rasmussen during the operations.

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THE DUCTLESS GLANDS AND HIBERNATION

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The phenomenon of hibernation is of great interest to the biologist. Its possible causes and physiologic processes have been the subject of many investigations. These experiments and observations have demonstrated many facts in regard to hibernation, and have especially emphasized the profound change in metabolism which takes place in the hibernating animal.

Many of the ductless glands certainly have considerable to do with the metabolic processes. As the changes in metabolism are so marked during the hibernating state, it was thought that possibly new insight into the function of some of the ductless glands, or a cause for hibernation, might be obtained by a study of these glands in a hibernating animal. Observations were carried out during the summer of 1914 and the winter of 1914–15, on a series of spermophilus tridecemlineatus (gophers). The number of animals studied was small and the results inconclusive. The publication of positive results by other workers (1) incited me to repeat the experiment, using a larger series of animals and observing control conditions more carefully. I shall report the complete study.

While many studies have been undertaken on different phases of hibernation (2) very few have been made with special reference to any of the ductless glands.

Gemelli (3) made a study of the hypophysis in 22 adult marmots. Some of these animals were sacrificed while in the hibernating state; others shortly after awakening, and the rest during the summer season. Each hypophysis was studied histologically. Gemelli did not find any modification in the nerve-lobe or in the posterior part of the glandular lobe. In the anterior part of the glandular lobe there were marked changes. These occurred only in the chromophile cells. During the summer these cells were accumulated in big clumps, but during hibernation they were enormously decreased. Shortly after the animal awakened many mitotic figures were found.

After our study was begun, Cushing and Goetsch (1) published an article dealing with the subject. Beside a general review of the clinical and experimental work done on the pituitary gland, the data submitted consisted of an histologic study of most of the ductless glands of seven woodchucks, three of which were hibernating and four active. The observations showed evidence of pluriglandular insufficiency in the dormant state, but the most striking change occurred in the anterior lobe of the pituitary gland. The latter consisted of a loss of characteristic cellular topography of the pars anterior, and, in the case of the individual cells, of a shrinkage of both the nuclear and protoplasmic substance of the cell with complete loss of the characteristic histologic picture of the active gland, namely, the differential staining qualities of the granular content with acid and basic dyes. On the basis of these observations Cushing and Goetsch (1) conclude that hibernation may be ascribed to the seasonal physiologic wave of pluriglandular inactivity. The essential role may perhaps be ascribed to the pituitary body not only because the most striking histologic change appears in this structure but also because deprivation of the secretion of this gland alone, of the entire ductless gland series, produces a group of symptoms comparable to hibernation.

In our experiments the spermophile was selected because of its great prevalence locally. While it is probably not as typical an example of the hibernating animal as the marmot, it does become profoundly lethargic. It was found to be a very satisfactory laboratory animal, being easily cared for and free from disease. However, several facts must be considered in adapting it to laboratory use. The old animals withstand operation very well but are prone to tear open their own or another's wound. To avoid this they must be kept in separate cages for a few days after operation and the wound should be sponged with a strong solution of iodin. The young during the first three or four months of life do not withstand operation very well. As the spermophiles do not all hibernate at the same temperature, it is advisable to maintain the temperature of the room in which several animals are kept together in one cage above the hibernating temperature; otherwise the active animals will kill those that are torpid. During the season of rut it is necessary to keep the males separate.

While the causative processes of hibernation are still unknown, there are environmental conditions which have long been considered important factors, namely, lowered temperature and a decreased food supply. To these may be added an intrinsic factor, the nutritional condition of the animal. I attempted to control these factors but found that in the spermophile they are of importance only within very wide limits. A spermophile will not become torpid immediately after eating, and withdrawal of food seems to be a factor in producing torpidity. However, it will hibernate even when it has access to a good food supply if the other factors are favorable. While changes of temperature are of the greatest importance, they are active only within a wide limit. Very rarely will an animal become torpid at a temperature higher than 24°C. and usually every animal will be lethargic when the temperature gets as low as 5°C. An animal does not always hibernate at the same temperature. It may be torpid at a certain temperature and a few days later active at a much lower temperature. Some animals after once becoming torpid remain in that condition for months. Others after becoming torpid may at various times be warm to the touch though not active. Still others pass through periods of torpidity and periods of activity when they will take food, without any apparent relation to temperature. In general, a very fat animal and a very thin one will become torpid sooner than an animal that is very active. It is thus seen that while temperature, food and the nutritional conditions are important factors, they are of value only when taken in consideration with the general phenomena of hibernation. In our study the temperature was found to be the only factor of any practical importance and it was of significance only when carefully controlled.

TABLE 1

*The temperature at which 100 spermophiles first became torpid**

CONDITION OF ANIMAL	TOTAL NUMBER	TEMPERATURE (CENTIGRADE)					
		15°	20°	10°	15°	5°	10°
		Number becoming torpid	Porcentage of total	Number becoming torpid	Percentage of total	Number becoming torpid	Percentage of total
Normal	60	40	66⅔	11	18⅓	9	15
Ovaries removed	10	6	60	1	10	3	30
Testicles removed	10	6	60	2	20	2	20
Adrenals removed	10	5	50	3	30	2	20
Thyroids removed	10	6	60	2	20	2	20

* Note that the removal of the ovaries, testicles, adrenals, and thyroids did not materially change the percentage of those becoming torpid at the different temperatures as compared with normal animals.

The animals were kept in several rooms so that each experiment could be carefully checked in regard to laboratory conditions. Some of the rooms were dark and could be maintained at any desired temperature. Other rooms were kept very warm, and others were allowed to change with the outside temperature. Usually when it was desired to make an animal hibernate, food was withdrawn, the animal was placed in a dark room and the temperature was gradually decreased. This approximated normal conditions, and as a rule, the method proved to be very satisfactory.

Two general methods were employed in investigating the possible relationship between the ductless glands and hibernation.

The first method consisted in a comparative histologic study of all the glands in active and torpid animals. The active animals were sacrificed by bleeding under light ether anesthesia. Specimens from active animals were obtained every few weeks throughout the year, thus eliminating the effect of seasonal variation. The glands of the hibernating animals were obtained at various times after the beginning of the lethargic condition. The torpid animals were sacrificed by bleeding; it was not necessary to anesthetize them. The specimens were fixed mainly in formalin, acetic Zenker and neutral formalin Zenker.

It is obvious that if any of the ductless glands are a causative factor in hibernation, only initial changes can be considered as offering any valid data in a study of this character, because after a long-continued period of hibernation, the changes might be the result and not the cause of the torpid state. For this reason the greatest attention was paid to the animals which had hibernated only a short time.

The second method consisted in removing certain of the ductless glands, and after various lengths of time determining whether the operated animals would become torpid in the same manner as the unoperated controls. If any of the glands of internal secretion are causative factors or inhibit the hibernating state, removal of these glands should vary the normal induction of torpidity. Of course this method could be used only in a study of the glands which can be surgically removed. Practically it was found that the sex glands, thyroids and adrenals were the only glands that could be studied by this method.

A study of all the tissues of the hibernating spermophile showed that there is one slight change which in general is common to most tissues. This change consists in a slight shrinking of the cell, with a decrease in both the distinctness of the cell outline and the intensity of the staining reaction. In additiion, there are marked fatty changes, especially in the liver, and congestion, especially in the spleen.

The ductless glands of the spermophile are very small. Because of this fact, several were lost or destroyed during the process of histologic technic. However, a sufficient number were obtained for a complete study.

The sex glands. With regard to hibernation there are minor differences between the male and female. In the wild state it seems that, in general, the male is the first to appear in the spring and the last to disappear in the fall. My observations seem to show that the female becomes torpid sooner than the male. The difference, however, is very slight.

The seasonal variation in the sex glands and accessory reproductive organs is well marked but need not concern us here except in regard to the fact that it takes place in animals that have been active all winter a little sooner than in those that have hibernated. Also as far as could be determined, captivity does not interfere with the sexual cycle except that it causes it to begin and terminate earlier in the season.

The testicle. The examination of the testicles at various periods of the year showed the changes occurring at various stages of the sexual cycle. These changes are very slight from the beginning of early fall to the beginning of rut.

In July, 1914, the testicles were removed from two spermophiles. The animals hibernated under eaxctly the same conditions as the normal controls during the winter of 1914–15. One of them was observed also during the fall of 1915 and no change was noted.

During the summer of 1915, twelve animals were castrated. These animals hibernated normally.

The ovaries. The ovaries show a marked seasonal variation. The luteal cells do not persist until the beginning of hibernation. The changes occurring in the ovary are those incident to the development of a large number of graafian follicles. Most of these follicles are developed before the period of hibernation, and during hibernation the changes are slight.

The ovaries were removed from two spermophiles in July, 1914. The animals hibernated normally during the winter of 1914–15 and the fall of 1915. During the summer of 1915 the ovaries were removed from twelve animals. All hibernated under the same condition as the normal animals.

TABLE 2

The effect of decrease in temperature on a series of normal and operated spermophiles

DATE	TIME OBSERVED A.M.	NORMAL ADULT MALE	NORMAL ADULT MALE	NORMAL ADULT FEMALE	NORMAL ADULT FEMALE	NORMAL YOUNG MALE	NORMAL YOUNG FEMALE	ADULT TESTICLES REMOVED	YOUNG TESTICLES REMOVED	YOUNG TESTICLES REMOVED	YOUNG TESTICLES REMOVED	ADULT OVARIES REMOVED	ROOM TEM. C.
11-11-15	8.00	active	active	active	active	active	active	active	active	active	active	active	25
11-12-15	8.00	active	sltly. torpid	active	active	active	active	active	active	active	active	active	20
11-13-15	8.00	active	torpid	active	torpid	active	active	active	active	active	active	sltly. torpid	19
11-14-15	9.00	active	torpid	sltly. torpid	torpid	torpid	torpid	sltly. torpid	sltly. torpid	active	active	torpid	18
11-15-15	8.00	active	torpid	sltly. torpid	torpid	torpid	torpid	sltly. torpid	torpid	active	active	torpid	17
11-16-15	8.00	active	torpid	sltly. torpid	torpid	torpid	torpid	sltly. torpid	torpid	sltly. torpid	active	torpid	16
11-17-15	8.00	active	torpid	torpid	torpid	torpid	torpid	sltly. torpid	torpid	sltly. torpid	active	torpid	15
11-18-15	8.00	active	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	active	torpid	10
11-19-15	8.00	active	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	active	torpid	10
11-20-15	8.00	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	sltly. torpid	torpid	5

DATE	TIME OBSERVED A.M.	ADULT OVARIES REMOVED	ADULT OVARIES REMOVED	ADULT OVARIES REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, ADRENALS REMOVED	ADULT FEMALE, ADRENALS REMOVED	ADULT FEMALE, ADRENA. REMOVED	ADULT FEMALE, ADRENALS REMOVED	ROOM TEM. C.
11-11-15	8.00	active	active	active	active	active	active	active	active	active	active	active	25
11-12-15	8.00	active	active	active	active	active	active	active	active	active	active	active	20
11-13-15	8.00	sltly. torpid	active	active	active	active	active	active	active	active	active	sltly. torpid	19
11-14-15	9.00	sltly. torpid	torpid	active	sltly. torpid	sltly. torpid	sltly. torpid	sltly. torpid	active	sltly. torpid	active	sltly. torpid	18
11-15-15	8.00	torpid	torpid	active	sltly. torpid	torpid	torpid	sltly. torpid	active	sltly. torpid	active	sltly. torpid	17
11-16-15	8.00	torpid	torpid	active	active	torpid	torpid	sltly. torpid	active	sltly. torpid	active	torpid	16
11-17-15	8.00	torpid	sltly. torpid	active	active	sltly. torpid	sltly. torpid	sltly. torpid	active	sltly. torpid	sltly. torpid	torpid	15
11-18-15	8.00	torpid	torpid	active	torpid	sltly. torpid	torpid	torpid	active	torpid	torpid	torpid	10
11-19-15	8.00	torpid	torpid	torpid	torpid	torpid	torpid	torpid	active	torpid	torpid	torpid	10
11-20-15	8.00	torpid	torpid	torpid	torpid	torpid	torpid	torpid	sltly. torpid	torpid	torpid	torpid	5

The thyroids. The histologic picture of the thyroid of the spermophile is very uniform. The acini are relatively large and filled with colloid; the cells very low or flat. The secretion granules are usually very few in number. I have not noticed any difference between the thyroids of active and hibernating animals.

The thyroid can be removed in the adult animal without the production of any changes. In July, 1914, the thyroids were removed from four spermophiles. These hibernated normally during the winter of 1914–15 and two were alive and hibernated during the fall of 1915. During the summer of 1915 the thyroids were removed from twelve animals. These hibernated under the same condition as the controls. In the animals operated on histologic examination showed that the thyroid was removed although it was impossible to state that all thyroid tissue had been taken out. Necropsies were performed on practically all the animals and in only one was any thyroid tissue found on macroscopic examination.

The parathyroids. The parathyroids are relatively large in the spermophile. They are found not only attached to the thyroids but also buried in the hibernating gland in the neck or anterior mediastinum. The histologic examination of these glands shows that in some animals they undergo very slight changes during hibernation. These changes consist in a decrease in their ability to take up the stains and a more irregular arrangement of the cells. In many hibernating animals they cannot be observed.

The thymus. A considerable amount of lymphoid tissue is always found buried in the substance of the hibernating gland in the anterior mediastinum. Hassall's corpuscles were noted in only a few of the animals examined. In some cases the staining ability of the lymphoid tissues and corpuscles of the gland from the hibernating animal seemed to be decreased. This decrease, however, was not uniform and so slight as not to warrant any positive statement.

The islands of Langerhans. The histologic picture of the pancreas of a hibernating spermophile does not differ essentially from that of a resting pancreas. The islands of Langerhans are large and well developed in the spermophile, and the A and B cells are well differentiated. The only changes noted in the islands of the hibernating animal were a very slight decrease in staining properties and an irregularity in the arrangement of the cells. This change is not uniform.

The adrenals. The adrenal of the spermophile is a small but perfect gland having a medulla and cortex with its three fairly well defined zones. This gland undergoes fairly definite and uniform seasonal changes. However, these changes have not been observed in all animals. The adrenal shows a gross increase in size in the spring and on histologic examination all the cells of both the cortex and medulla are found to be swollen. The main change takes place in a stratum of cells which are located in the inner part of the columns of the zona fasciculata. These cells are greatly swollen and very large in the spring. As the season advances the size of the adrenal decreases. Histologically all the cells appear to become smaller but the major changes consist in a marked decrease in the cells of the inner portion of the zona fasciculata and a vacuolization of the cells of the zona granulosa and the upper portion of the zona fasciculata. During hibernation the latter changes seem to be accentuated but the lipoid content does not seem to change greatly. The medullary cells shrink very slightly during hibernation.

I was surprised to discover that the adrenals could be removed from the spermophile without producing immediate death, except in a small percentage of cases. In a few instances the animals died typical deaths from adrenal insufficiency. As a rule the animal recovered from the operation and either became perfectly normal or more frequently lost weight and strength gradually, dying several weeks after removal of the last gland. In some cases necropsy revealed a hypertrophied accessory adrenal but in most cases a diligent search with the bichromate method and histologic examination of suspicious particles did not reveal any adrenal tissue. (4) This probably does not mean that the spermophile can live without adrenal tissue or that another gland vicariously takes up its function, but that scattered bits of adrenal tissue which were too small to be found without serial section of the whole animal were enough to keep the animals alive for various lengths of time. However, it is of particular importance in the study that, at any event, the animal in which the adrenals were removed had a minimum amount of functionating adrenal tissue. This was amply proved by the fact that most of the animals became thin and weak and died.

The adrenals were removed from a series of animals in July, 1914. Five of them lived and hibernated during the winter of 1914–15. Twelve additional adrenalectomized animals were studied during the fall of 1915 and were found to hibernate under the same condition as the controls.

While these results show that the adrenals undergo fairly constant seasonal changes, they are just as much indicative of increased functional activity during the period of rut as of decreased activity during hibernation. It would appear that the adrenals do not bear any causal relation to hibernation for two reasons. First, the adrenalectomized animals, which certainly had only a minimum amount of adrenal tissue, hibernated normally. Second, the histologic changes noted in the hibernating animals were progressive, being found to some extent in the active animals in the autumn, and quite as marked in the animals which were sacrified during the winter and which had not hibernated owing to the high temperature of the room.

The pituitary. The spermophile has a well-formed pituitary gland. It does not differ from the corresponding glands of other species except that the differential staining properties of the cells of the anterior glandular portion are not very marked. I have not been able to note any seasonal variation in the pituitary. In some of the pituitary glands of hibernating spermophiles I have found the same changes observed by Cushing and Goetsch in the woodchuck. These consist in an almost complete loss of the normally rather slight differential staining qualities of the granular content of the cells to acid and basic dyes, a decrease in the size of the cells and an irregularity in the cellular grouping. However, these changes are not constant. In several hibernating spermophiles I have not been able to distinguish any changes in the pituitary glands. In many, the changes are very slight. No mitotic figures were seen.

The inconstancy of the changes is proved very easily by a study of the glands having controls. I am not able to see any difference between the pituitaries of Gophers 225 and 227; 114 and 270, or 271 and 272.

The results of the study were as follows:

1. The sex glands undergo a definite seasonal variation but certainly do not play any part in the cause of the hibernating state or undergo any specific changes due to the torpid condition.
2. I have been unable to determine any changes in the thyroids of the hibernating animal, and the fact that animals in which the thyroids were removed hibernated normally tends to show that these glands are not factors of significance in hibernation.
3. The very slight and inconstant changes noted in the parathyroids do not warrant any positive conclusion.
4. The thymus does not appear to undergo any uniform change.

5. The islands of Langerhans undergo changes so slight as not to justify a positive conclusion.

6. The adrenal glands undergo a fairly definite seasonal variation. The different changes are most marked during the winter and during the period of rut. However, the adrenals do not appear to be a specific factor in hibernation because the same changes were noted in animals sacrificed during the winter which had not been allowed to hibernate, and removal of the adrenals, which certainly reduces the adrenal substance to a minimum, did not in any way change the hibernating ability of the animal.

7. Some of the pituitary glands of the hibernating animals showed definite changes, but these changes were not constant.

CONCLUSION

It is impossible to state that some of the ductless glands are not of importance in relation to hibernation. New methods of investigation may demonstrate this relationship. However, the results of this study do not justify the assumption of any theory ascribing the phenomena to a lack of function of all or any one of the ductless glands.

BRIEF RECORDS OF MOST OF THE SPERMOPHILES WHOSE DUCTLESS GLANDS WERE STUDIED

A. Active animals

No.

105 Female; weight 100 grams; captured April, 1915; pregnant; fasted for five days. Sacrificed May 5, 1915.

106 Male; weight 135 grams; captured April, 1915; fasted for five days. Sacrificed May 5, 1915.

108 Female; captured with two of her young in June, 1914. Very savage. Was kept in warm room during the winter of 1914–15, but hibernated when the temperature was allowed to decrease below 20°C. Sacrificed May 21, 1915.

147 Female; weight 125 grams; captured June, 1915. Sacrificed June 12, 1915.

148 Female; captured June, 1915. Sacrificed June 12, 1915.

149 Male; weight 143 grams; captured June, 1915. Sacrificed July 20, 1915.

174 Female; weight 144 grams; captured June, 1915. Sacrificed July 20, 1915.

175 Male; weight 134 grams; captured June, 1915. Sacrificed July 20, 1915.

192 Female; captured July, 1915. Had lactated. Sacrificed August 3, 1915.

198a Female; captured in the spring of 1915. Sacrificed August 14, 1915.

199a Female; captured in the spring of 1915. Sacrificed August 14, 1915.

204 Male; captured season 1915. Sacrificed September 14, 1915.

240 Female; very fat; weight 180 grams; captured early in the spring of 1915. Sacrificed September 24, 1915.

No.

241 Male; captured in the spring of 1915. Sacrificed September 24, 1915.

246 Female; captured in the spring of 1914. Both ovaries removed July 10, 1914. During the winter of 1914–15 was allowed to hibernate for short periods, but was active most of the time. Sacrificed September 21, 1915. Weight at time of death, 95 grams.

142 Female; weight 135 grams; captured May, 1915. Both ovaries removed June 3, 1915. Animal became slightly torpid at times during the autumn months, but was kept in a very warm room and did not hibernate. Sacrificed November 10, 1915.

146 Female; weight 128 grams; captured May, 1915. Uterus removed June 11, 1915. Sacrificed July 19, 1915. Weight at this time, 135 grams.

251 Female; weight 101 grams; captured July, 1914. Uterus and left ovary removed October 5, 1915. Sacrificed October 26, 1915.

225 Male; weight 110 grams; born in the spring of 1915. Animal placed in hibernating room without food at 12.00 m., November 11, 1915. Remained very active until November 20, when it seemed slightly torpid. Rectal temperature 20°C. Sacrificed at this time. (Control for Spermophile 227.)

114 Female; weight 120 grams; captured June, 1915. Animal was well supplied with food and kept in warm room. Did not become torpid while in captivity. Sacrificed February 2, 1916. (Control for Spermophile 270).

268 Female; weight 55 grams; born in the spring of 1915. Animal had never hibernated. Food withdrawn but room kept warm, February 3, 1916. Sacrificed February 5, 1916. (Control for Spermophile 267.)

269 Female; weight 50 grams; born in the spring of 1915. Animal had never hibernated. Sacrificed February 24, 1916. (Control for Spermophile 220.)

271 Female; weight 140 grams; captured July, 1915 Had lactated that season. Did not hibernate while in captivity. Sacrificed February 28, 1916. (Control for Spermophile 272.)

275 Male; weight 105 grams; captured in the spring of 1915. Spleen removed July, 1915. Was not allowed to become torpid while in the laboratory. Sacrificed March 27, 1916. Genital tract showed the marked hypertrophy and hyperemia of beginning rut. (Control for Spermophile 274.)

278 Female; weight 85 grams; captured July, 1915. Had lactated in the spring of 1915. Did not hibernate during the winter of 1915–16. Sacrificed April 11, 1916. At necropsy the animal showed pregnancy of a few days' duration.

284 Female; weight 65 grams; captured July, 1915. Born in the spring of 1915. Did not hibernate during the winter of 1915–16. Sacrificed April 21, 1916.

B. Hibernating animals

	Time hibernating	
267	12 hours	Female; weight 60 grams; born in the spring of 1915; kept under observation and it is certain that it had never become torpid until placed in the hibernating room without food February 4, 1916, at 4.00 p.m. It became torpid about 10.00 p.m. Was sacrificed February 5, at 10.00 a.m. Rectal temperature 11°C.

No.	*Time hibernating*	
252	1 day	Fat female; captured in the spring of 1915. November 8, 1915 at 4.00 p.m. placed in room with temperature of 14° C. with no food. Had not hibernated previously during the season. Remained active until 10.00 am., November 9. Sacrificed November 10 at 10.00 a.m. Rectal temperature 14°C.
253	2 days	Fat female; captured in the spring of 1915. November 8, 1915, placed in room to hibernate, with no food. Did not become torpid for several hours. Sacrificed November 12, after hibernating for two days. Temperature of room at this time 9°C. Rectal temperature 11°C.
255	3 days	Fat female; captured in the spring of 1915. Placed in hibernating room, without food. Remained active for several hours. Sacrificed November 18, 1915, after hibernating three days. Rectal temperature 12°C.
127	4 days	Male; weight 150 grams; captured in the spring of 1915. Placed in hibernating room without food; remained there less than twenty-four hours. Sacrificed December 2, 1915, after being torpid four days. Rectal temperature 14°C. Room temperature 12°C.
227	5 days	Male; weight 108 grams; born in the spring of 1915. Animal placed in hibernating room, without food, at 12.00 m., November 11, 1915. Became torpid first November 16. Sacrificed November 20, at 10.30 a.m. Rectal temperature 10°C.
254	6 days	Very fat female; captured in the spring of 1915. When placed in hibernating room without food, became torpid very quickly. Sacrificed November 15, 1915, after hibernating six days. Rectal temperature 8.5°C. Room temperature for the six days varied from 5° to 10°C.
226	7 days	Male; weight 96 grams; born in the spring of 1915. September 9, 1915, both testes removed. Animal recovered from operation and increased in weight. Placed in hibernating room without food; sacrificed December 15, 1915, after hibernating seven days. Rectal temperature 9.5°C. Weight when sacrificed 110 grams.
132	8 days	Female; weight 115 grams. Captured May, 1915. Placed in hibernating room without food; sacrificed December 11, 1915, after being torpid eight days. Rectal temperature 15°C.
259	9 days	Female; weight 100 grams; captured in the spring of 1915. Placed in hibernating room without food; sacrificed December 17, 1915, after hibernating nine days. Room temperature at this time 0°C. Rectal temperature 5°C.
256	10 days	Very fat female; captured in the spring of 1915. Placed in hibernating room without food; sacrificed November 20, 1915, after hibernating ten days. Rectal temperature 11.5°C.
260	15 days	Female; weight 85 grams; captured in the spring of 1915. Animal had been torpid continuously for fifteen days, although it had been torpid for a short time earlier in the season. Did not have access to food; sacrificed January 1, 1916. Rectal temperature 12°C.

No.	*Time hibernating*	
170	20 days	Female; weight 145 grams; captured in the spring of 1915. Animal did not have access to food; sacrificed January 15, 1916, after being torpid approximately twenty days. Rectal temperature 14°C.
258	25 days	Male; weight 150 grams; captured in the spring of 1915. Did not have access to food; sacrificed December 11, 1915, after hibernating twenty-five days. Rectal temperature 12.5°C.
257	30 days	Very fat female; weight 195 grams; captured in the summer of 1915. Had lactated that season. Did not have access to food; sacrificed December 11, 1915, after being torpid thirty days. Rectal temperature 12.5°C.
117	35 days	Male; weight 165 grams; captured in the spring of 1915. Animal had access to food. Had been torpid thirty-five days. Sacrificed January 10, 1916. Rectal temperature 14°C.
261	40 days	Very fat male; weight 150 grams; captured in the spring of 1915. Had access to food. Sacrificed January 12, 1916, after hibernating forty days. Rectal temperature 2.5°C.
221	45 days	Female; weight 85 grams; born in the spring of 1915. Both thyroids removed September 20, 1915. Placed in hibernating room without food; sacrificed January 13, 1916, after being torpid continuously for forty-five days. Rectal temperature 6°C.
121	50 days	Male; weight 180 grams; captured May, 1915. Sacrificed January 13, 1916. It had been hibernating for fifty days without access to food. Had shown some signs of activity for a short time during this period but did not become active. Rectal temperature 5°C.
263	75 days	Female; weight 100 grams; captured in the spring of 1915. Sacrificed January 12, 1916, after hibernating seventy-five days without access to food. This animal was slightly active a few times during this period. Rectal temperature 12°C.
272	80 days	Female; weight 90 grams; captured in the spring of 1915. Sacrificed February 28, 1916. Had been hibernating without access to food for eighty days. Rectal temperature 19°C. Daily observations were not taken on this animal.
262	100 days	Male; weight 95 grams; captured in the spring of 1914. Hibernated at intervals during the winter of 1914–15. Food was withdrawn October 1, 1915, and the room allowed to approximate outside temperature. Animal immediately became torpid. Sacrificed January 12, 1916, after hibernating about one hundred days. Animal rather thin. Rectal temperature 11°C. Daily notes not taken.
266	125 days	Female; captured June, 1914. Hibernated when kept in cold during the winter of 1914–15. Placed in hibernating room and food withdrawn the latter part of September, 1915. Sacrificed January 27, 1916, after hibernating about one hundred and twenty-five days. Rectal temperature 17°C. Daily observations were not made.

No.	*Time hibernating*	
270	150 days	Female; captured May, 1914. Hibernated at intervals during the winter of 1914–15. Was placed in hibernating cage, without food, October 1, 1915. Daily observations were not taken, but it is known that the animal was torpid most of the time, although at intervals it was slightly warm to the touch. Sacrificed February 28, 1916. Quite torpid; rectal temperature 21°C. Very thin. Weight 75 grams.
220	Indefinite	Male; weight 93 grams; born in the spring of 1915. Both thyroids removed September 20, 1915. Hibernated at intervals throughout the winter. Had access to food. Sacrificed February 24, 1916. Quite torpid. Rectal temperature 20°C. Very thin; weight 50 grams. No thyroid tissue found. Daily observations not taken.
228	Indefinite	Male; weight 82 grams; born in the spring of 1915; both testicles removed September 21, 1915. Hibernated at intervals throughout winter. Had access to food. Sacrificed February 28, 1916. Quite torpid. Rectal temperature 20°C. Weight 65 grams. Daily observations not taken.
135	Indefinite About 40 days	Female; weight 122 grams; captured May, 1915. Spleen removed May 28, 1915. Animal recovered from operation and became fat. December 13, 1915, placed in hibernating room with food. In the attempt to determine the reaction of the hibernating animal to excessive cold, it froze to death January 13, 1916. Tissues of all organs were well preserved.
118	Indefinite About 45 days	Female; weight 105 grams; captured in the spring of 1915. Placed in hibernating room with food December 3, 1915. In studying effect of excessive cold upon hibernating animals, it was frozen to death January 13, 1916. Tissues were in excellent preservation.
264	Indefinite About 40 days	Female; weight 95 grams; captured in the spring of 1915. Placed in hibernating room with food December 3, 1915. Was frozen to death during the study of the effect of excessive cold upon hibernation, January 13, 1916. Tissues well preserved.
265	Indefinite About 40 days	Male; weight 95 grams; captured in the spring of 1915. Placed in hibernating room with food December 3, 1915. Was frozen to death during the attempt to determine the effect of excessive cold upon hibernation, January 13, 1916. Tissue well preserved.

C. Active immediately after hibernation

237 Male; born in the spring of 1915; right adrenal removed September 23, 1915. Had access to food throughout winter. Hibernated at intervals. Sacrificed March 11, 1916. Animal very active at this time. Very poor weight—50 grams.

210 Female; captured in the spring of 1915; right adrenal removed September 15, 1915. Had access to food throughout the winter. Hibernated at rare intervals. Sacrificed March 11, 1916. Animal very fat; weight 110 grams.

No.

126 Female; captured in the spring of 1915. December 12, 1915, placed in hibernating room without food. While daily observations were not made, it is known that the animal was very torpid although two or three times it was found to be active. At 9.00 a.m., March 11, 1916, animal was torpid; rectal temperature 15°C. Animal was placed in warm room and at 10.00 a.m. was very active. Sacrificed at 3.00 p.m. Weight 75 grams.

273 Female; weight 95 grams. Captured late in the spring of 1915. Had lactated during the season. Hibernated at intervals during the winter of 1915–16. Had access to food. Was hibernating March 11, when it was placed in a warm room, and remained active. Sacrificed March 18.

231 Female; weight 200 grams. Captured in the spring of 1915. Ovaries removed September 22, 1915. Hibernated at intervals throughout winter 1915–16. Had access to food. Sacrificed March 18, 1915; weight at this time 100 grams.

274 Male; weight 85 grams. Captured in the spring of 1915. Hibernated during the winter of 1915–16. Had access to food. Was torpid until March 11, when its rectal temperature was 20°C. When placed in a warm room, it remained active. Sacrificed March 27.

276 Female; weight 105 grams. Captured in the spring of 1915; hibernated during the winter of 1915–16. Had access to food. Active after March 11. Sacrificed March 27.

190 Female; weight 106 grams. Captured in the spring of 1915. July 23, 1915, complete removal of both thyroids. Animal hibernated during the winter of 1915–16. Had access to food. It was placed in a warm room March 11, and thereafter was never noted to be torpid. Sacrificed April 4; weight at this time 83 grams.

273 Male; weight 145 grams. In excellent condition. The first animal caught in the spring of 1916. Captured and sacrificed April 11, 1916. Marked hyperemia and hypertrophy.

280 Female; weight 120 grams. Captured July, 1915. Had lactated during the season of 1915. Hibernated during the winter of 1915–16. Had access to food. Was not torpid after March 10. Sacrificed April 14, 1916.

281 Female; weight 145 grams. Captured and sacrificed April 14, 1916.

282 Male; weight 170 grams. Captured and sacrificed April 14, 1916.

283 Female; weight 110 grams. Captured and sacrificed April 17, 1916.

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(4) Vincent in Internal Secretion and the Ductless Glands, 1913, 116, states that he has never been able to find chromophile bodies in the spermophile.

THE REGULATION OF THE BLOOD VOLUME AFTER INJECTIONS OF SALINE SOLUTIONS[1]

STUDIES OF THE PERMEABILITY OF CELLULAR MEMBRANES. I

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The volume of the blood is one of the important constants of the body, the maintenance of which constitutes a condition of health. With widely varying fluid intake, there must frequently be a tendency toward disturbance of this equilibrium and toward an alteration in the volume of the blood. The mechanism by means of which a constant blood volume is normally maintained is, therefore, of very general physiological interest.

Attention has again been directed to these subjects by recent investigations. Boycott ('13) found that, in normal rabbits, the original volume of the blood was restored in a short and fairly constant interval after intravenous injection of Ringer's solution. On the other hand, rabbits rendered nephritic by uranium nitrate showed a partial failure of the blood volume to return to normal under the same experimental procedure. Chisholm ('14 a) found a similar incomplete regulation of the blood volume in rabbits with nephritis induced by potassium chromate; and likewise ('14 b) in normal rabbits after the injection of extract of kidneys from animals with uranium or chromate nephritis.

The present series of investigations, suggested by the work of Boycott and Chisholm, was undertaken with the object (1) of studying further the phenomena described by them in normal and nephritic rabbits; (2) of determining whether a similar disturbance of the regulation of the blood volume was to be found in rabbits suffering from tartrate nephritis; and (3) of extending these studies to the dog, in order to follow the flow of lymph from the thoracic duct in normal and nephritic animals after injections into the blood stream.

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[1] The data in this paper are taken from the dissertation presented by L. Jean Bogert for the degree of Doctor of Philosophy, Yale University, 1916.

The general plan of experimentation employed in the work of Boycott ('13 and '14) and Chisholm ('14, a and b) has been followed in the present investigation. After the concentration of hemoglobin in the blood was determined, a known quantity of fluid was forced into the circulation under pressure as rapidly as possible. Immediately after the injection and at regular intervals thereafter the concentration of hemoglobin was measured and the relative blood volumes were calculated from these figures.

Technique

Ringer's solution,[2] warmed to 38°C., was used for the injections of saline solution. The fluid was usually introduced into the ear vein in rabbits and into the femoral vein in dogs. The injections were made under pressure and generally at the rate of about 50 cc. per minute. Samples of blood were taken at first from the vessels of the rabbits' ear but it was found that such samples did not give constant results so that all blood samples were later taken from the carotid artery in rabbits. In the case of dogs, the blood samples were uniformly taken from the femoral artery. Only a few drops of blood were required for hemoglobin determination. These were caught in depressions in a paraffin plate and determinations made at once to avoid loss of water by evaporation. Rabbits were anaesthetized with ether only, but dogs generally received a subcutaneous injection of 10 mg. of morphine sulphate and one of atropine sulphate per kilogram of body weight one hour previous to anaesthetization with ether. Hemoglobin determinations were made according to the method suggested by Haldane,[3] the accuracy of the standard used having been checked by determinations of the oxygen content of blood, made by the Haldane-Barcroft method.

Calculation of the original blood volume of rabbits was made on the assumption (Boycott '13) that they have about 50 cc. of blood per kilogram of body weight. They usually received injections of this amount of fluid; but the dogs only half as much or 25 cc. per kilogram of body weight. In some cases the animal was given a second or third injection. An illustrative example of the method of calculating the relative blood volumes follows: if the original blood volume is taken as 100 per cent and if the content of hemoglobin was 60 per cent before injection and 40 per cent after injection, the relative blood volume after injection would be $\frac{60}{40}$ x 100 = 150 per cent.

The accuracy of such calculations depends, obviously, on the assumption that the amount of hemoglobin in the circulation is constant. Experimental data have been published recently to show that the number of red blood corpuscles may be increased in high altitudes[4] or when work,[5] causing an appreciable amount of perspiration, is done. Lamson[6] also found increased red corpuscle content of the blood as a result of partial asphyxiation or fright. Increase in the number of red blood corpuscles may, of course, take place without an increase of the amount of hemoglobin in the circulation; but, in order to test the validity of the method used, a number of control experiments were made.

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[2] This solution contained 0.9 per cent sodium chloride, 0.024 per cent calcium chloride, 0.042 per cent potassium chloride and 0.03 sodium bicarbonate.

[3] Haldane: Journ. of Physiol., 1901, xxvi, 497.

CONTROL EXPERIMENTS

Hemoglobin content in the same animal at intervals. The concentration of hemoglobin in the blood was determined at half hour intervals in four normal rabbits and the results are given in the table below.

TABLE 1

Hemoglobin content in the same animal at intervals

	HEMOGLOBIN IN PER CENT							
	0 hr.	½ hr.	1 hr.	1½ hr.	2 hr.	2½ hr.	3 hr.	3½ hr.
Rabbit A................	54	53	54	53				
Rabbit B................	70	69	70					
Rabbit C................	68	68	69	68	68	69	68	69
Rabbit D................	73	74	74	73	74			

Comparison of hemoglobin and total solid content of the blood after injections of saline solution. The ordinary injection of 25 cc. of Ringer's solution per kilogran of body weight was given to a number of dogs, samples of blood being taken before and after the injection and at intervals later. The percentage of hemoglobin in the blood was determined and, in addition, the percentage of water was also ascertained by drying to constant weight at 100 to 110°C. The results of these experiments are given in the following table:

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[4] Schneider and Havens: This Journal, 1915, xxxvi, 380.

[5] Boothby and Berry: This Journal, 1915, xxxvii, 378.

[6] Lamson: Journ. of Pharm. and Exper. Therap., 1915, vii, 169.

TABLE 2

Comparison of the hemoglobin and total solid content of the blood after injections of saline solutions

TIME	EXPERIMENT 43		EXPERIMENT 60		EXPERIMENT 61		EXPERIMENT 65	
	Hemoglobin	Total solids	Hemoglobin	Total solids	Hemoglobin	Total solids	Hemoglobin	Total solids
	per cent		*per cent*		*per cent*		*per cent*	
Before injection	44	13.6	77	20.7	83	22.8	70	21.9
After injection								
Immediately	31	10.7	55	16.0	66	18.5	55	17.6
10 minutes					74		60	
20 minutes					78		63	
25 minutes	40	12.7						
30 minutes			70	20.5	83	22.9	63	20.5
40 minutes	40	13.1			84		64	
50 minutes					83		64	
60 minutes			76	21.3	84	22.7	64	20.4

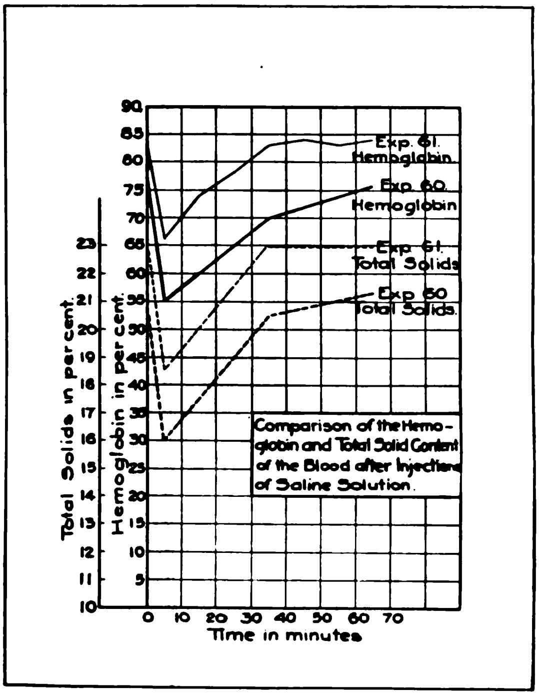

Fig. 1

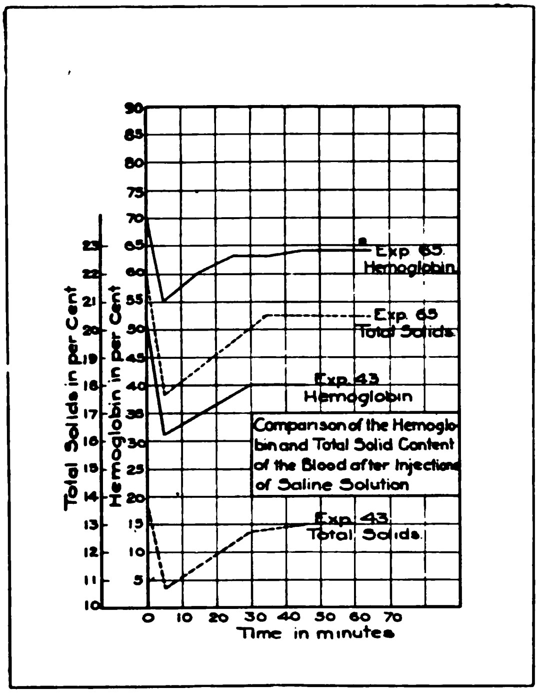

Fig. 2

The first set of experiments (table 1) clearly shows that the hemoglobin does not vary in amount in individual animals, at least during periods of several hours duration. The second series of experiments (table 2) establishes the fact that the hemoglobin is a true measure of the dilution of the blood, since the results obtained by this method are in close accord with those obtained by the determination of the total solid content of the blood. When the figures in table 2 are represented graphically (see figs. 1 and 2), the parallelism of the hemoglobin and total solid curves is seen to be almost perfect. Under the conditions of these experiments, then, it is safe to conclude that the amount of hemoglobin in the circulation does not alter materially and, therefore, it can be used as a measure of the dilution of the blood.

EXPERIMENTS WITH RABBITS

Under normal conditions. A specimen protocol is given below. The results of thirteen such experiments are summarized in table 3.

Experiment 21. Rabbit ♀, normal, weighing 2140 grams. Injection of 100 cc. of Ringer's solution into the ear vein in 2 minutes; blood samples from carotid artery.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	37	100
After injection		
Immediately	23	160
5 minutes	32	116
10 minutes	35	106
15 minutes	37	100
20 minutes	37	100

No fluid in pleural cavity; about 3 cc. in peritoneal cavity.

TABLE 3

Regulation of the blood volumes in normal rabbits after injections of saline solution

EXPERIMENT NUMBER	RELATIVE BLOOD VOLUMES AFTER						
	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.
1		131	128	109	100	100	100
2		121	112	105	100	101	100
3			115	104	98	100	100
4	150	133	113	107	101	100	100
5	136	114	116	105	103	100	
6	152	139	134	131	117	113	101
7	141	120	114	109	107	101	100
11	151	127	118	106	103	104	
12	143	121	114	105	100	100	
13	137	114	106	102	100	100	
21	160	116	106	100	100		
22	130	114	107	104			
23	159	130	123	110	105		
Average..	146	121	116	107	103	102	100

From these figures it appears that a great deal of the fluid introduced leaves the blood stream during the period of the injection. The more rapid the introduction of the solution the less is the amount of liquid able to leave the circulation during this period. In experiment 3, where the duration of the injection was fifteen minutes, the blood volume was found to be only 15 per cent above normal at the close of the injection, while the greatest increase in relative blood volume noted was in experiment 21, where the solution was introduced in two minutes. It is, however, to be remembered that the calculated original blood volume is only approximate and that undoubtedly some animals received more fluid than others, in proportion to their original blood volume.

In spite of some individual variations, the figures show a satisfactory agreement. The initial dilution caused by the injection varies somewhat, due largely to the causes outlined in the preceding paragraph; but the length of time required for the blood volume to return to normal is about twenty-five minutes in almost every case. It will be observed that the fluid leaves the blood stream most rapidly in the earlier moments after the injection; i.e., the stimulus to rid the blood of the excess of fluid is strongest when the excess is greatest.

In general, then, it was found that, after injection of a quantity of saline solution equal to the calculated blood volume, into normal rabbits, the excess of fluid disappears from the circulation rapidly and the original blood volume is restored in a short time, usually not more than thirty minutes.

After ligation of the kidneys. In order to determine the influence of the kidney factor in the regulation of the blood volume in normal rabbits, the kidneys were ligated at the hilum and injections of Ringer's solution were then made. In some of these experiments the blood volume returned to normal in the same length of time as in normal rabbits / with the kidneys active. Others showed a very slight delay. Of the two protocols given here, that of experiment 19 is an example of the former class and experiment 17 of the latter.

Experiment 19. Rabbit ♀, both kidneys ligated, weighing 1780 grams. Injection into ear vein of 100 cc. of Ringer's solution in 2 minutes; blood samples from carotid artery.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	40	100
After injection		
Immediately. 1 min	28	142
5 minutes	34	118
10 minutes	36	111
15 minutes	38	105
20 minutes	40	100
25 minutes	40	100
30 minutes	39	102
45 minutes	39	102
55 minutes	40	100

Killed one hour after injection. Kidneys found completely ligated; no fluid in pleural or peritoneal cavities.

Experiment 17. Rabbit ♀, both kidneys ligated, weighing 2200 grams. Injection into ear vein of 100 cc. of Ringer's solution in 2 minutes; blood samples from carotid artery.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	55	100
After injection		
Immediately	39	141
5 minutes	48	114
10 minutes	50	110
15 minutes	51	108
20 minutes	52	106
25 minutes	53	104
30 minutes	54	102
35 minutes	53	104
50 minutes	54	102
60 minutes	55	100

Killed one hour after injection. Kidneys found completely ligated; no fluid in pleural and peritoneal cavities.

From these experiments it seems that ligation of the kidneys produces no marked effect on the regulation of the blood volume. Boycott ('13 and '14) and Chisholm ('14 a and b), ligated the ureters just above the bladder in all their normal animals and their results are very similar to those of the experiments recorded in table 3, in which healthy rabbits with active kidneys were used. Moreover, in experiments 1 to 13, the urine was collected by compression of the bladder at half hour or hour intervals; and there was no connection whatever between the amount of urine secreted and the rate of restoration of the blood volume to normal. The amount of urine secreted varied widely. In some animals a volume of urine equal to or greater than the volume of the injected fluid, was secreted in four or five hours, while in others only about one-fourth of the injected fluid had been excreted after three or four hours. In this connection, it is interesting to note that some of the rabbits which showed a poor excretion of the fluid by the kidneys had diarrhoea the following day. At the end of thirty minutes, however, when the blood volume had in practically every case returned to normal, only 5 to 28 per cent of the injected volume had left the body as urine. Autopsy showed no fluid in the body cavities. It seems clear, therefore, that the tissues act as a reservoir for the excess of fluid, which later leaves the body gradually, and that the activity of the kidney plays only a small rôle in the regulation of the blood volume after intravenous injection of salt solutions.

After starvation and lack of water. To ascertain whether the previous condition of the tissues had any effect on the regulation of the blood volume, three rabbits were kept without food or water for three days before injection. These experiments were also designed as controls for the experiments with nephritic rabbits, which frequently refuse food and water.

Experiment 10. Rabbit ♀, weighing 1660 grams, deprived of food and water for three days. Injection of 100 cc. of Ringer's solution in 2 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME	URINE
	per cent		*cc.*
Before injection	56	100	
After injection			
Immediately	44	127	
5 minutes	46	123	
10 minutes	51	110	
15 minutes	54	104	
20 minutes	56	100	
25 minutes			8
1 hour			3
1½ hours			2
2 hours			5
2½ hours			12
3 hours			13
			43

TABLE 4

Regulation of blood volume after starvation and lack of water

EXPERIMENT NUMBER	RELATIVE BLOOD VOLUMES AFTER					
	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
8	188	138	119	111	106	100
9	123	119	109	106	103	
10	127	123	110	104	100	

The figures in the above table indicate that a condition of food and water deprivation has little or no influence on the rate with which injected fluids leave the blood stream. Probably the capacity of the tissues to act as a reservoir for excess of fluid is so great that the quantities of liquid dealt with in these experiments have no appreciable effect on the mechanism for maintaining constant blood volume. In experiments 9 and 10 there seems some support for the idea that tissues poor in water draw the excess of fluid from the blood stream more rapidly, since the relative blood volumes immediately after injection are considerably below the average figure for normal rabbits (see table 3). In experiment 8, however, the initial dilution is unusually high, which cannot be accounted for by the rapidity of the injection, as the rate of injection was the same in all three experiments.

In uranium nephritis. The rabbits were rendered nephritic by subcutaneous injection of 10 mg. of uranium nitrate per kilogram of body weight, the same dosage as that used by Boycott ('13 and '14). Although the characteristic incomplete recovery of the normal blood volume after injections into the blood stream is usually apparent on the second day after the injection of the uranium nitrate, it may be absent, in case the nephritis is not sufficiently advanced; and it is much more marked on the fourth or fifth day. As anuria sets in about the third day of the nephritis and death frequently results by the fifth day, the fourth day after administration of the uranium nitrate was found to be the best time for experimental purposes.

Experiment 36.[7] Rabbit ♀, nephritic, weighing 2000 grams.

July 19—Subcutaneous injection of 10 mg. of *uranium nitrate* per kilogram.

July 22—About 6 cc. of urine which gave tests for protein and sugar. Animal was in poor condition, lethargic and breathing irregularly.

Injection of 100 cc. of Ringer's solution in 1 minute, 40 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	65	100
After injection		
Immediately	35	186
5 minutes	43	151
10 minutes	49	133
15 minutes	53	123
20 minutes	51	127
25 minutes	54	120
30 minutes	53	123

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[7] These and the following protocols have been selected as examples from a large number of duplicate experiments.

Injection II of 100 cc. of Ringer's solution in 1 minute, 45 seconds.

After injection		
Immediately	34	191
5 minutes	40	163

Rabbit died suddenly, with foaming at mouth and nose, ejection of a colorless watery fluid and convulsive struggles. Kidneys were very pale, with no edema or hypertrophy. The bladder contained 8 cc. of urine, the peritoneal cavity contained no fluid but there were 10 cc. of clear fluid in the pleural cavity.

Experiment 35. Rabbit ♀, nephritic, weighing 2200 grams.

July 19—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

July 21—100 cc. of urine which gave a flocculent precipitate on heating and reduced Benedict's solution.

Injection of 110 cc. of Ringer's solution in 1 minute 45 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	62	100
After injection		
Immediately	38	163
5 minutes	49	126
10 minutes	53	117
15 minutes	53	117
20 minutes	55	113
25 minutes	55	113
30 minutes	55	113
35 minutes	55	113

Injection II of 110 cc. of Ringer's solution in 2 minutes.

After injection		
Immediately	34	182
5 minutes	46	135
10 minutes	50	124
15 minutes	50	124
20 minutes	53	117
25 minutes	53	117
30 minutes	55	113

Kidneys were pale, with no edema and the bladder was empty. There was no fluid in the pleural cavity, while the peritoneal cavity contained 20 cc. of clear fluid which clotted rapidly.

Experiment 36 shows a distinct disturbance of the mechanism for regulation of the blood volume associated with the nephritic condition. Not only is the initial dilution caused by the injection greater than in normal rabbits, indicating the inability of the excess of fluid to leave the circulation as rapidly, but at the end of thirty minutes when the injected fluid would have completely disappeared from the blood stream of a normal rabbit, 23 per cent of it is still present in the blood stream of the nephritic animal. Moreover, all the fluid removal took place during the first fifteen minutes after the injection. During the last fifteen minutes of the experiment, it had apparently been impossible for the organism to remove any more liquid from the circulation. An attempt was made to determine the effect of a second injection but the animal died.

The effects noted under experiment 36 are reproduced in experiment 35, although they are not so pronounced. This is probably due to the fact that the animal employed had been subjected to the influence of uranium nitrate for a period of two days only. A second injection was here successful and was followed by increased initial dilution of the blood and a slower rate of removal of the excess of fluid. It is interesting to note that the same extent of recovery of the original blood volume (113 per cent relative blood volume) took place after each injection and that further removal of fluid seemed to be impossible.

In tartrate nephritis. Since Chisholm ('14 a) had found the same disturbance of the regulation of the blood volume in rabbits with chromate nephritis, it seemed of interest to ascertain whether a similar condition prevailed in rabbits suffering from a nephritis induced by sodium tartrate. The procedure suggested by Underhill, Wells and Goldschmidt[8] was followed. The rabbits were given no food for two days prior to receiving a subcutaneous injection of 2 grams of sodium tartrate. They were allowed plenty of water and drank freely. On the day following the tartrate injection the animals were almost or completely anuric and were used for experimentation.

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[8] Underhill, Wells and Goldschmidt: Journ. of Exper. Med., 1913, xviii, 322.

Experiment 27. Rabbit ♀, nephritic, weighing 3000 grams.

June 2—Placed in cage with plenty of water but without food.

June 4—Subcutaneous injection of 2 grams of *sodium tartrate.*

June 5—Weight, 2600 grams. Had drunk 140 cc. of water in the last twenty-four hours. Animal was anuric but had diarrhoea.

Injection of 130 cc. of Ringer's solution in 2 minutes 15 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	38	100
After injection		
Immediately	25	152
5 minutes	30	126
10 minutes	30	126
15 minutes	30	126
20 minutes	31	122
25 minutes	30	126
30 minutes	30	126
35 minutes	30	126
50 minutes	31	122
60 minutes	30	126

Injection II of 130 cc. of Ringer's solution in 3 minutes.

After injection		
Immediately	20	190
5 minutes	25	152

Rabbit died, with evident respiratory failure. Kidneys were pale, hypertrophied and watery; bladder was empty. Peritoneal cavity contained about 10 cc. fluid and there was a very little clear fluid in the pleural cavity. Some subcutaneous edema about abdominal region.

From the results of this experiment it is evident that tartrate nephritis has the same effect on the regulation of the blood volume shown in uranium and chromate nephritis. The severity of the nephritis is shown by the fact that even after injection of 260 cc. of Ringer's solution, no urine was secreted. Sixty per cent of the injected fluid left the circulation during the period of injection and 20 per cent during the next five minutes. However, 20 per cent of the solution introduced still remained in the blood stream, and no further removal of this excess of fluid took place in the following 50 minutes. Death, due to respiratory failure, resulted after a second injection.

The average figures obtained from all the experiments on normal and nephritic rabbits are given in table 5 and these figures were used in plotting the curves shown in the accompanying diagram.

TABLE 5

Experiments on regulation of blood volume in rabbits

Average figures

	RELATIVE BLOOD VOLUMES AFTER							
	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.
Normal	146	121	116	107	103	102	100	
Nephritic (uranium) Injection I	157	133	127	119	118	116	116	117
Nephritic (tartrate) Injection I	152	123	120	123	115	117	115	115
Nephritic (uranium) Injection II	187	149	129	129	127	127	125	
Nephritic (tartrate) Injection II	165	144	134	130	130	125	123	126

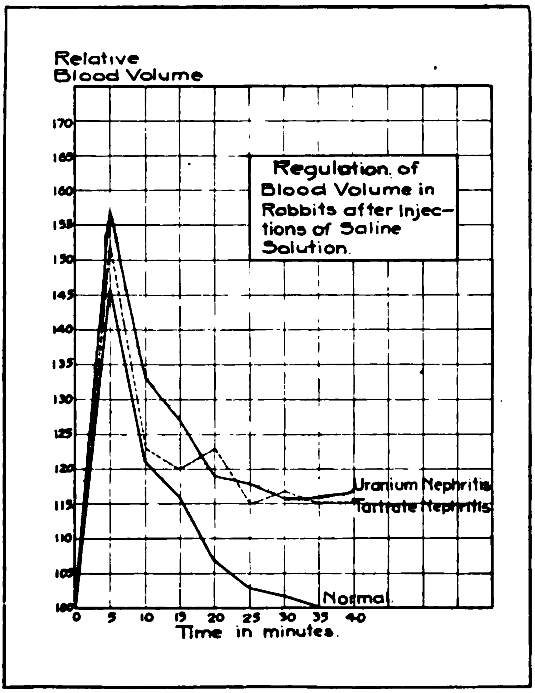

Fig. 3

Discussion of results. The experimental evidence given above, in conjunction with the work of Boycott ('13 and '14) and Chisholm ('14, a and b), shows clearly that fluid leaves the blood and passes into the tissues less readily in nephritic than in normal rabbits. It remains to be considered which of the factors concerned in the adjustment of the blood volume is so altered by the nephritis as to hinder the passage of fluid from the blood to the tissues. No change in the osmotic pressure of the blood would be caused by the injection of Ringer's solution and the conditions of blood pressure would undoubtedly be equally affected in both the nephritic and normal animals. The experiments on rabbits with ligated kidneys and after food and water deprivation show that, with the experimental procedure employed in these investigations, the factors of kidney activity and condition of the tissues have little apparent effect on the regulation of the blood volume. The possibility of the retention of water by the nephritic animals causing a dilution and lowered osmotic pressure of the tissue fluids has not been excluded; but

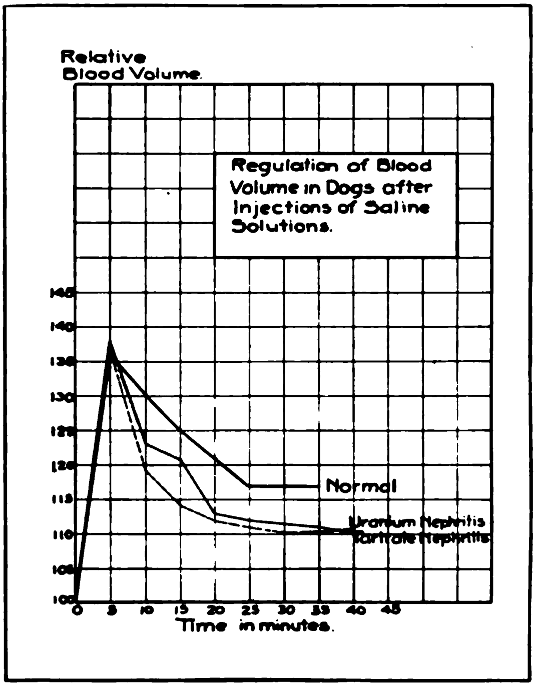

Fig. 4

this factor would seem to be more than balanced by the retention of solids in anuric conditions, especially as the nephritic animals frequently refused water. An alteration of the permeability of the blood vessel walls seems the only adequate explanation of the changed conditions prevailing in the nephritic animals. It is, therefore, concluded that the condition of nephritis effects an alteration of the permeability of the walls of the capillaries of the general circulation in such a way as to hinder the passage of fluid from the blood to the tissues.

To test the limits of tissue capacity. As was pointed out in the discussion of the experiments on rabbits with ligated kidneys, the injected saline solution does not leave the blood through the activity of the kidney nor is it to be found as transudates into the body cavities or in the alimentary canal. It must, therefore, be absorbed by the tissues. The following experiments were carried out with a view to ascertaining how much fluid a rabbit with ligated kidneys could dispose of and still keep the blood volume normal. It was also hoped to determine whether the tissues of nephritic rabbits showed a diminished capacity to hold water.

Experiment 70. Rabbit ♂, both kidneys ligated, weighing 2000 grams. Injection of 400 cc. of Ringer's solution in 9 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	61	100
After injection		
Immediately	37	165
5 minutes	51	120
20 minutes	58	105
30 minutes	61	100
40 minutes	61	100

Both kidneys found completely ligated; peritoneal cavity with 10 cc. clear fluid; pleural cavity with no fluid; walls of the alimentary tract highly edematous; no respiratory distress.

Experiment 67. Rabbit ♀, both kidneys ligated, weighing 2420 grams. Injection of 414 cc. of Ringer's solution in 7 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	58	100
After injection		
Immediately	24	242
10 minutes	32	181

Respiratory difficulty was so great that injection was discontinued before four times the blood volume (480 cc.) was injected. Dyspnoea continued unabated till death. On autopsy both kidneys were found completely ligated; peritoneal cavity with only 1 to 2 cc. of fluid; pleural cavity moist; lungs very congested but with no hemorrhages; viscera of the peritoneal cavity highly edematous.

Experiment 70 shows that a normal rabbit, with kidneys ligated, can dispose of a quantity of fluid equal to four times the calculated blood volume of the animal. A large amount of water is taken up by the walls of the intestines but the amount secreted into the intestinal lumen is small. There is no general edema, only a small quantity of fluid in the peritoneal cavity and none in the pleural cavity.

Experiment 67 shows that the limit of the capacity of the tissues is reached at approximately four times the blood volume of the animal, as in this experiment, this quantity, which was successfully disposed of in experiment 70, caused death when injected a little more rapidly. Injections of saline solution equal to two and three times the original blood volume (see table 6, experiments 66 and 68) were well borne, but death resulted when an injection equal to four times the blood volume was given to an animal, which had previously received a quantity of fluid equal to two times its blood volume. Death was preceded in each case by symptoms of great respiratory distress and the ejection of fluid from the nose. It is evident, from the figures in table 6, that, in order to have the animal dispose of the fluid, it is important that the rate of injection be so regulated that the liquid is not introduced much more rapidly than it can leave the circulation. Thus, in experiment 68, there was a greater initial dilution and more difficulty in disposing of the excess of fluid than in experiment 70, where the volume of the solution introduced was greater but where the rate of injection was slower.

Experiment 73. Rabbit ♀, nephritic, weighing 1680 grams.

December 10—Subcutaneous injection of 10 mg. of *uranium nitrate* per kilogram.

December 14—Urine for previous twenty-four hours, 35 cc. which gave a heavy precipitate on heating and faint reduction of Nylander's solution. Weight 1600 grams.

Injection of 320 cc. of Ringer's solution in 11 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	46	100
After injection		
Immediately	26	177
10 minutes	38	121
20 minutes	37	124
30 minutes	38	121
40 minutes	38	121
50 minutes	38	121
60 minutes	38	121

Kidneys were pale and edematous; bladder was empty; pleural cavity contained about 35 cc. of rapidly clotting, clear fluid, while pleural cavity contained about 3 cc. of clear fluid.

Experiment 74. Rabbit ♀, nephritic, weighing 1700 grams.

December 12—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

December 15—No urine voided in previous twenty-four hours.

December 16—Urine, 5 cc. containing sugar and protein. Animal used for experimentation.

Injection of 340 cc. of Ringer's solution in 12 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	48	100
After injection		
Immediately	24	200
10 minutes	34	141
20 minutes	35	137
30 minutes	35	137
40 minutes	35	137
50 minutes	35	137

Kidneys were very pale and inflamed, with much edema; bladder was empty; the pleural cavity contained 5 cc. and the peritoneal cavity 45 cc. of clear fluid. which clotted rapidly; walls of the intestine edematous.

Experiment 73 furnishes evidence that the capacity of the tissues to hold water is no less in nephritic than in normal conditions. It was thought that the nephritic rabbits might not be able to dispose of large injections as well as of smaller ones, a greater excess of fluid remaining in the blood the larger the injection. In experiment 73, however, the relative blood volume at the end of the experiment is no higher than that usually seen after injections one-fourth as large. In experiment 74 the final relative blood volume is slightly higher but there is reason to conclude from the autopsy that the nephritis was of a more acute type than in experiment 73.

It has been shown that saline solutions equal to four times the blood volume can be completely removed from the circulation within thirty minutes in normal rabbits. In nephritic animals, under the same experimental procedure, the extent of fluid removal is equal to that observed when the amount of fluid introduced was equivalent to the blood volume. It is, therefore, concluded that the injection of saline solution employed in these experiments, that is, a volume equal to that of the blood, is sufficient to produce marked alterations in blood volume without introducing excessive quantities of fluid.

TABLE 6

Capacity of tissues in normal rabbits

	RELATIVE BLOOD VOLUME AFTER						
	Injection	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Experiment 66. Two times the normal blood volume injected in 4 minutes	146	119	113	102	100	100	
Experiment 68. Three times the normal blood volume injected in 6 minutes	193	153	126	121	114	107	102
Experiment 70. Four times the normal blood volume injected in 9 minutes	165	120	105	100	100		
Experiment 67. Four times the normal blood volume injected in 7 minutes	242	181					

TABLE 7

Capacity of tissues in nephritic rabbits

	RELATIVE BLOOD VOLUME AFTER						
	Injection	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Experiment 73. Four times the normal blood volume injected in 11 minutes	177	121	124	121	121	121	121
Experiment 74. Four times the normal blood volume injected in 12 minutes	200	141	137	137	137	137	

EXPERIMENTS WITH DOGS

In order to follow the lymph flow after intravenous injections of saline solutions, and to determine whether the above observations made on rabbits are applicable as well to dogs, the experiments made on normal and nephritic rabbits were repeated on dogs. Narcosis was produced by a subcutaneous injection of 10 mg. of morphine sulphate and 1 mg. of atropine sulphate per kilogram of body weight; and ether was given whenever necessary. A fistula of the thoracic duct having been made, the lymph was collected in five minute periods. Ringer's solution (25 cc. per kilogram) was injected, the remainder of the procedure being the same as in the experiments on rabbits.

Under normal conditions and after ligation of the kidneys. In experiment 18 the curve of the blood volume after saline injections is very different from that seen in normal rabbits. Instead of returning to normal within half an hour after the injection, the blood volume remains above normal throughout the experiment. A second injection produces a similar but more marked effect. Ligating the kidneys is without influence on the regulation of the blood volume.

The lymphagogic action of the saline injections is noticeable, the quantity of lymph being increased four fold or more. The amount of fluid which leaves the blood and passes into the thoracic lymphatic system is, however, small in proportion to the size of the injection. In experiment 18, after the injection of 500 cc. of Ringer's solution, the lymph collected amounted to only 123 cc. or about 25 per cent of the injected fluid. Most of the fluid had left the blood but was evidently absorbed and held back by the tissues.

Experiment 18. Dog ♂, normal, weighing 10.25 kilograms.

8.30 a.m.—Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

9.30 a.m.—Anaesthetized with ether. Cannula in thoracic duct.

Injections of Ringer's solution into right femoral vein. Blood samples from left femoral artery.

REMARKS	LYMPH		BLOOD		RELATIVE BLOOD VOLUME
	Time	Interval flow	Time	Hemoglobin	
		cc.		per cent	
	10.41				
	10.46	1.5			
	10.51	1.65			
10.59–11.01, injection of 250 cc. Ringer's solution	10.56	1.75	10.54	93	100
	11.01	2.10	11.01	72	129
	11.03	5.4			
	11.07	9.9	11.06	77	121
Deep breathing and cries	11.12	10.6	11.11	80	116
	11.17	6.4	11.16	82	113
	11.22	5.6	11.21	84	111
	11.27	4.5	11.26	85	109
	11.32	4.0	11.31	84	111
	11.37	3.6	11.36	84	111
	11.42	3.9	11.41	84	111
	11.47	3.7			
	11.52	3.3	11.51	84	111
	11.57	2.8			
12.02–12.25, operation of ligating kidney at hilum	12.02	2.8	12.01	85	109
	12.25		12.30	86	108
12.30–12.32, injection of 250 cc. Ringer's solution	12.30	1.3	12.32	68	137
	12.35	2.1	12.37	75	124
	12.40	9.2	12.42	77	119
	12.45	6.7	12.47	79	117
	12.50	6.9	12.52	82	113
	12.55	5.6	12.57	80	116
	1.00	4.4	1.02	80	116
	1.05	4.3	1.07	82	113
	1.10	3.9	1.12	83	112
Deep breathing and cries	1.15	3.7			
	1.20	3.4			
	1.25	2.8	1.22	87	107
	1.30	3.3			
	1.35	3.0	1.32	86	108

Experiment 26. Dog ♂, nephritic, weighing 12 kg.

June 2—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kilo.

June 3—Urine 560 cc. Specific gravity 1.030. Reaction acid. Heavy flocculent precipitate on heating. Heavy sediment on standing. Very few casts, round cells very prominent, no blood cells. Benedict's solution strongly reduced.

Injection of 6.7 mg. *uranium nitrate* per kg.

June 4—Weight 10 kg. Drank freely of water. Urine 420 cc. Sediment on standing.

9 a.m.—Injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kg.

10 a.m.—Anaesthetized with ether. Cannula inserted in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE
	Time	Interval flow	Time	Hemoglobin	Blood volume
		cc.		*per cent*	
	10.22				
	10.27	2.7	10.26	63	100
10.36–10.38 Injection I of 250	10.32	2.9			
cc. Ringer's solution	10.39		10.38	46	137
Lymph grows turbid	10.44	3.5	10.43	52	121
Lymph very turbid	10.49	3.7	10.48	55	114
	10.54	4.0	10.53	55	114
	10.59	4.8	10.58	56	112
Lymph watery and clear	11.04	5.0	11.03	57	111
	11.09	3.5	11.08	54	116
	11.14	2.9	11.13	55	114
11.23–11.24½ Injection II of 250	11.19	2.2	11.18	55	114
cc. Ringer's solution	11.25		11.25	42	150
Lymph clear	11.30	6.4	11.30	47	134
Lymph turbid	11.35	3.8	11.35	52	121
	11.40	4.0	11.40	54	116
	11.45	3.5	11.45	54	116
	11.50	3.6	11.50	54	116
	11.55	4.1	11.55	55	114
Lymph clear	12.00	3.1	12.00	55	114
	12.05	3.0	12.05	56	112
	12.10	4.3	12.10	56	112
	12.15	3.4	12.15	57	110
12.23–12.27 Injection III of 500	12.20	3.4	12.20	56	112
cc. Ringer's solution			12.27	40	157
	12.32	10.7	12.32	44	143
Lymph turbid	12.37	8.5	12.37	45	140
	12.42	6.8	12.42	46	137
	12.47	5.6	12.47	46	137
	12.52	5.6	12.52	48	133
	12.57	9.3	12.57	48	133
	1.02	4.2	1.02	49	128
	1.07	5.4	1.07	51	123
	1.13	4.6	1.13	52	121
	1.18	1.1			
	1.23	.6	1.21	50	126
No flow of lymph—due to wrong position of cannula—no clot.	1.28	1 drop	1.26	52	121

Kidneys were pale, hypertrophied and watery; bladder contained a little less than 50 cc. of urine; peritoneal cavity contained about 5 cc. of fluid.

In uranium nephritis and in tartrate nephritis. Uranium nephritis was induced by subcutaneous injections of 6.7 mg. of uranium nitrate per kilogram of body weight on two successive days and the dogs were used for experimental purposes on the third day.[9] Tartrate nephritis was produced in the same manner as in the rabbits.

Experiment 32. Dog ♀, nephritic, weighing 8.2 kg.

June 14—Placed in cage without food. Drank freely.

June 16—Subcutaneous injection of 8 g. *sodium tartrate.*

June 17—8.30 a.m. Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

Anaesthetized with ether at 9.45 a.m. Cannula inserted in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE
	Time	Interval flow	Time	Hemoglobin	Blood volume
		cc.		*per cent*	
	10.57				
Lymph flow uniform	11.02	0.8	11.02	75	100
11.03–11.04 Injection I of 200	11.07	2.5	11.05	56	134
cc. Ringer's solution	11.12	7.1	11.10	63	121
	11.17	4.5	11.15	66	114
Lymph clears	11.22	4.2	11.20	68	110
	11.27	2.7	11.25	70	107
Animal cries	11.32	3.3	11.30	70	107
	11.37	2.5	11.35	69	108
11.43–11.44 Injection II of 200	11.42	2.2	11.40	70	107
cc. Ringer's solution	11.47	7.0	11.45	54	139
	11.52	14.0	11.50	60	125
	11.57	9.5	11.55	60	125
	12.02	7.0	12.00	62	121
	12.07	5.1	12.05	64	117
	12.12	5.2	12.10	66	114
	12.17	3.8	12.15	67	112
12.23–12.25 Injection III of 400	12.22	3.9	12.20	65	115
cc. Ringer's solution	12.27	12.0	12.25	43	174
	12.32	13.7	12.31	55	136
	12.37	8.1			
	12.42	8.4	12.40	63	121
	12.47	8.0	12.45	63	121
	12.52	8.4	12.50	63	121
Respiration increased	12.57	10.6	12.55	63	121
	1.02	8.5	1.00	65	115

Kidneys were pale but normal in size and without edema. Bladder contained 30 cc. of urine, which was very light in color and gave a strong protein test; there was no fluid in either the pleural or peritoneal cavities.

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[9] MacNider: Journal of Med. Res., 1913, xxviii, 403.

These experiments display a condition similar to that in the normal dog and in the nephritic rabbit following saline injections. There is uniform inability to restore the blood volume to normal and it will be noted that the excess of fluid in the circulation is about the same in dogs after two injections of 25 cc. per kilogram as in nephritic rabbits after a single injection of 50 cc. per kilogram. The lymph flow varies widely in individual dogs but is not markedly different in nephritic and normal animals. These observations are shown in graphic form in the charts which follow.

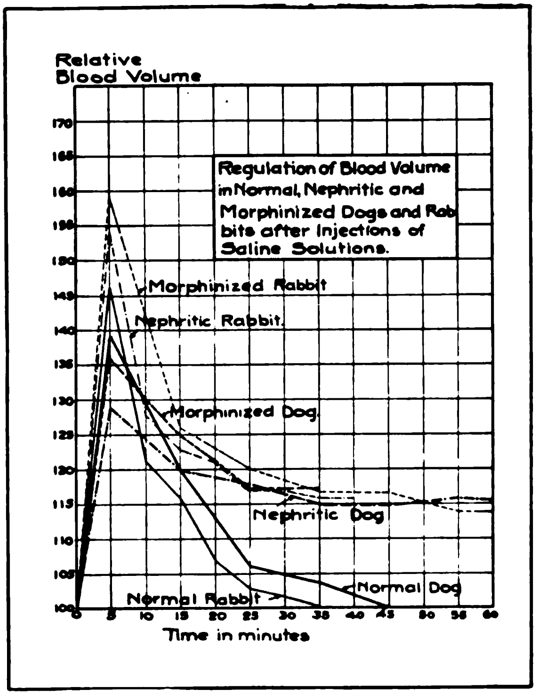

Fig. 5

THE INFLUENCE OF MORPHINE AND ATROPINE UPON BLOOD VOLUME REGULATION FOLLOWING INJECTIONS OF SALINE SOLUTIONS

In attempting to explain these conflicting data, it seemed hardly probable that difference in species would account for this wide divergence in physiological behavior. The most important distinction in experimental procedure appeared to be that the dogs were given a subcutaneous injection of 10 mgm. of morphine sulphate and one of atropine sulphate per kilogram one hour previous to anaesthetization, while the rabbits were not. Investigations were, therefore, made to determine whether this factor was responsible for the different results obtained in the experiments on dogs.

In normal dogs. The results of such experiments are seen in the following illustrative protocol.

Experiment 71. Dog ♂, normal, weighing 8 kg. No morphine and atropine injection given. Animal anaesthetized with ether. Injection of 200 cc. of Ringer's solution in 1 minute 30 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	80	100
After injection		
Immediately	61	131
10 minutes	70	114
20 minutes	75	107
30 minutes	80	100

Experiment 71 demonstrates that normal dogs which have not received morphine and atropine are able to completely restore the blood volume to normal after saline injections, behaving in the same manner as rabbits under similar circumstances. This is confirmed by the results of four other similar experiments. On the other hand, Experiments 43 and 65 (see table 2) argue against this conclusion, since, in these experiments, the blood volume had not returned to normal after periods of forty minutes and one hour respectively, although the dogs had received no morphine-atropine injection and were supposedly normal. As no proof was obtained that these animals were actually normal and as one of them (experiment 43) was obviously in very poor condition, a fact which was further evidenced by the abnormally low content of hemoglobin and total solids in the blood, it is possible that they were either spontaneously nephritic or were suffering from some other condition which produces a similar effect on the regulation of the blood volume. The fact that none of the animals, which received morphine and atropine were able to completely restore their normal blood volume after saline injections, while this was accomplished by five dogs, which had not received morphine-atropine injections, clearly indicates that this factor is responsible for the inability to restore normal blood volume.

In normal rabbits. In order to complete the evidence on this point, three rabbits were given injections of morphine and atropine sulphates, the same amounts per kilogram of body weight being subcutaneously administered and the same length of time being allowed to intervene before the injection of the saline solution as in the experiments on dogs. The rabbits showed themselves less sensitive to morphine than the dogs, for the narcotic effect of that drug was not noticeable. Nevertheless, the effect on blood volume after injections of saline solution was the same as that observed in dogs. This is readily seen in the protocol which follows.

Experiment 63. Rabbit ♂, with morphine and atropine injection. Weight, 1800 grams.

9 a.m.—Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram. 10 a.m.—Bladder emptied and animal etherized. Injection of 90 cc. of Ringer's solution in 1 minute 30 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	49	100
After injection		
Immediately	29	169
10 minutes	40	122
20 minutes	43	114
30 minutes	42	114
40 minutes	42	114
50 minutes	43	114
60 minutes	42	114

Bladder contained 5 cc. of urine; no fluid in the pleural cavity; 2 to 3 cc. fluid in the peritoneal cavity.

The subcutaneous injection of morphine and atropine sulphates results, therefore, in an ineffective regulation of the blood volume similar to that produced by nephritis. It seems probable that the morphine-atropine injection decreases the permeability of the capillary walls either directly by acting on the blood vessels themselves or indirectly by producing some injury to the kidney which results in this decreased capillary permeability. Whether this effect is due to the morphine or to the atropine or to their combined action, remained to be determined and the results of this investigation are seen in the following experiments.

Experiment 79. Rabbit ♂, with atropine injection, weighing 2220 grams.

1.15 p.m.—Subcutaneous injection of 1 mg. atropine sulphate per kilogram.

2.20 p.m.—Animal anaesthetized with ether.

Injection of 110 cc. of Ringer's solution in 2 minutes 10 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	66	100
After injection		
Immediately	48	137
10 minutes	58	114
20 minutes	66	100
30 minutes	66	100

Experiment 80. Rabbit ♂, with morphine injection, weighing 2420 grams.

2.20 p.m.—Subcutaneous injection of 10 mg. morphine sulphate per kilogram.

3.20 p.m.—Anaesthetized with ether. The most marked narcotic effect of morphine noted in rabbits was seen in this experiment. The animal was rendered stupid before ether was given and after anaesthetization no further ether was required in the course of an hour.

Injection of 120 cc. of Ringer's solution in 2 minutes 20 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	69	100
After injection		
Immediately	48	144
10 minutes	58	119
20 minutes	61	113
30 minutes	61	113
40 minutes	62	111
50 minutes	62	111
60 minutes	62	111

Experiments 79 and 80 prove that the failure of the blood volume to return to normal, after injections of saline solutions, produced in both dogs and rabbits by the injection of mophine and atropine sulphates, is the result of the action of the morphine sulphate.[10] Experiment 80 gives evidence that this effect of the morphine is not due to the decreased blood pressure, which it causes, since, in that event, the effect should be more pronounced when morphine is given alone than when it is given with atropine, which tends to nullify the action of the morphine on blood pressure.

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[10] Since the above statement was written, Kleiner and Meltzer (Proc. Soc. Exp. Biol. and Med., 1916, xiii, 142) have indicated that, after intravenous injections of dextrose, morphine retards the disappearance of the excess of sugar from the circulation, as shown by a persistent hyperglycemia.

In nephritic dogs. Experiments have been cited to show that the regulation of the blood volume after injections of saline solution is the same in normal dogs and rabbits without morphine administration. Injections of morphine have been shown to cause inability to restore the original blood volume after injections of saline solution in both dogs and rabbits. The same effect has been produced in rabbits by inducing a nephritic condition. If this partial recovery of original blood volume after injection were found to be the result of a nephritic condition in dogs, the analogy would be complete and the deductions made from experiments on one species might be regarded as applicable to the other. As, unfortunately, all the earlier data on nephritic dogs were taken from animals which had received morphine, no conclusions could be drawn from these experiments. A number of dogs were, therefore, rendered nephritic with uranium nitrate and injected with Ringer's solution as in the earlier procedure except that no morphine-atropine injection was given. When these animals were used for experimentation on the second day of the nephritis, little or no defect in the regulation of the blood volume was found. In all later experiments, therefore, the dogs were used on the fourth day of the nephritis, as was the case with the rabbits with uranium nephritis, and results entirely comparable with those noted in nephritic rabbits were obtained. A specimen protocol follows.

Experiment 77. Dog, nephritic. Weight 10.5 kg.—male.

January 18—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kilogram.

January 19—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kilogram. 320 cc. urine, containing sugar but no protein.

January 20—560 cc. urine, containing sugar and protein.

January 21—220 cc. urine, containing sugar and protein.

January 22—140 cc. urine, containing neither sugar nor protein.

Injection of morphine and atropine omitted. Ether anaesthesia only.

Injection of 250 cc. of Ringer's solution in 1 minute 50 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	72	100
After injection		
Immediately	56	128
10 minutes	59	122
20 minutes	62	116
30 minutes	62	116
40 minutes	64	113
50 minutes	62	116
60 minutes	62	116

Kidneys were congested, with great edema; bladder was empty; there was no fluid in the body cavities.

It thus appears that the existence of a condition of acute nephritis results in an inability of the animal to completely restore its blood volume to normal after injections of saline solution, in dogs as well as in rabbits.

In one experiment, although the characteristic effect of the nephritis on the regulation of the blood volume was present, it was much less marked than usual. This is probably accounted for by the fact that the dog used was young.[11] It seems somewhat more diffcult to produce an acute nephritis in dogs than in rabbits. The diuretic effect of the uranium is more marked and anuria does not set in till later. While

TABLE 8

Regulation of blood volume in normal, nephritis and morphinized dogs and rabbits after injections of saline solution

Average figures

	RELATIVE BLOOD VOLUMES AFTER											
	Injection	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min.	60 min.
Normal rabbits	146	121	116	107	103	102	100					
Normal dogs	139		120		106		104		100		100	100
Nephritic rabbits	154	128	123	121	117	117	116	116				
Nephritic dogs	129		120		118		115		115		116	115
Morphinized rabbits	159		126		120		117		117		114	114
Morphinized dogs	136	130	125	121	117	117						

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[11] MacNider, Journal of Pharmacology and Experimental Therapeutics, iv, 491, 1913.

it is not necessary that anuria should set in before the deficient regulation of the blood volume is noted, it is essential that the nephritis should have progressed to a certain degree of acuteness.

It seems probable, therefore, that, since the regulation of the blood volume has been shown by experimentation to be the same in both dogs and rabbits in all cases where similar conditions prevailed, conclusions drawn from experiments on either species may be considered as applicable to the other species.

SUMMARY OF THE MORE IMPORTANT CONCLUSIONS

The regulation of the blood volume in normal animals is both rapid and efficient; for complete restoration of the original blood volume takes place within thirty minutes after the intravenous injection of a quantity of saline solution equal to the calculated blood volume of the animal.

The activity of the kidneys is not essential to this regulation of the blood volume, as it occurs even after ligation of these organs.

In conditions of nephritis, inability to restore the blood volume to normal after injections of saline solution usually manifests itself. Experiments on animals with *uranium nephritis* confirm the results of Boycott in this respect. New experiments on animals with *nephritis induced by sodium tartrate* reveal a similar incomplete regulation of the blood volume under these circumstances.

Morphine sulphate, administered subcutaneously in large doses, *produces an effect on the regulation of the blood volume* comparable with that resulting from a condition of nephritis.

The factor of species is probably of little importance in studies on this regulation of the blood volume, since dogs and rabbits react alike when the conditions are identical.

Restoration of the blood volume to normal after intravenous injections of saline solutions is not primarily effected by the transfer of the excess of fluid from the blood vessels to the thoracic lymphatic system. The tissues act as a reservoir for this fluid.

The capacity of the tissues of rabbits to absorb fluid is approximately four times the normal blood volume of the animal.

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THE ACTION OF SALINE-COLLOIDAL SOLUTIONS UPON THE REGULATION OF BLOOD VOLUME[1]

STUDIES OF THE PERMEABILITY OF CELLULAR MEMBRANES. II

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There are few references in the literature relative to intravenous injections of colloidal solutions. In explanation of the small changes in arterial pressure caused by the injection of large quantities of blood, von Regéczy ('85) offered the suggestion that the injected fluid leaves the circulation. As proof of this, he cited an increased lymph flow following such injections and the higher specific gravity of the blood after blood of a lower specific gravity had been injected. Magnus ('01) also made large injections of blood and reported that 30 to 56 per cent of the injected fluid left the circulation within an hour although no diuresis resulted. Roth ('99) found that, when a protein solution (dialyzed horse serum) was placed in the peritoneal cavity, water was first drawn into the blood on account of the low osmotic pressure of the protein solution, but that later, when the osmotic pressures were equalized, protein began to diffuse into the blood and salts to diffuse out. He concluded that water in the form of a colloidal solution can pass through the walls of the blood vessels and that these walls are semipermeable for colloids as well as crystalloids.

Fischer and Hogan ('12) made intravenous injections of gelatin solutions and reported that these were not diuretic and that, therefore, high arterial pressure was maintained for a longer time after such injections than after injections of saline solutions. It was suggested that gelatin solutions might advantageously replace ordinary saline solutions for injection in cases of shock where it is desirable to keep the blood pressure high for a considerable period of time (Hogan '15). In the experiments of Roger and Garnier ('13) Locke's solution, to which gum or gelatin had been added, was found to leave the blood vessels less readily than plain Locke's solution. That gelatin solutions disappear from the blood stream less rapidly than saline solutions and less rapidly in nephritic than in normal rabbits were the conclusions reached by Boycott ('14) as a result of his experiments on the injection of colloidal solutions in rabbits.

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[1] The data in this paper are taken from the dissertation presented by L. Jean Bogert for the degree of Doctor of Philosophy, Yale University, 1916.

EXPERIMENTAL

The experiments to be detailed were carried out in the same manner as those in the preceding paper except that the Ringer's solution injected contained approximately 2 per cent of gelatin. In making this solution care was taken to follow the directions given by Hogan ('15) for making gelatin solutions for injection in man and evidently the colloidal nature of the gelatin was not injured.

EXPERIMENTS WITH RABBITS

Under normal conditions. Of the six normal rabbits, which received injections of gelatin solution, three were able to restore their blood vol-

Experiment 50. Rabbit ♀, normal, weighing 1700 grams. Injection of 85 cc. of gelatin solution in 2 min. 5 sec.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	43	100
After injection		
Immediately	30	143
5 minutes	34	126
10 minutes	38	113
15 minutes	39	110
20 minutes	39	110
25 minutes	39	110
35 minutes	41	105
40 minutes	43	100
45 minutes	43	100
50 minutes	43	100
60 minutes	43	100
70 minutes	43	100

In the bladder were 50 cc. of urine, secreted during the experiment, which contained no coagulable protein but gave a strong biuret test. There were about 4 cc. of fluid in the peritoneal cavity and a small amount in the pleural cavity.

ume to normal in the same length of time as after a saline injection, or with only a slight delay, while three rabbits showed some difficulty in disposing of the injected fluid, from 11 to 27 per cent of it being still present in the circulation after an hour. Experiment 50 is an example of the first group and experiment 46 represents the second group.

Experiment 46. Rabbit ♀, normal, weighing 3100 grams. Injection of 150 cc. of gelatin solution in 4 min. 20 sec.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	63	100
After injection		
Immediately	38	166
5 minutes	39	161
10 minutes	42	150
15 minutes	41	154
20 minutes	48	131
25 minutes	48	131
30 minutes	50	126
35 minutes	50	126
40 minutes	52	121
45 minutes	54	117
50 minutes	54	117
60 minutes	55	115
70 minutes	54	117

In the bladder were 30 cc. of urine, containing no coagulable protein or reducing substances but giving a strong biuret test. There was no fluid in the pleural cavity and only 3 to 4 cc. in the peritoneal cavity.

The bladder was emptied previous to the injection, in these experiments, and the urine secreted during the period of observation was collected. The amount secreted in one hour after the injection varied from 6 to 59 per cent of the injected fluid, with an average figure of 27 per cent. The data collected on urine excretion in normal rabbits after saline injections show that 9 to 53 per cent of the injected fluid, or an average of 28 per cent was excreted by the kidneys in the hour following the injection. From these experiments, therefore, it appears that after introduction of colloidal solutions fluid is excreted to the same extent as with saline solutions.

Boycott ('14) found that gelatin leaves the circulation and that the quantity lost varies, in a general way, with the quantity of liquid expelled. In the above experiments, the samples of urine taken an hour after the injection were all found to show no coagulable protein but to give a strong biuret test. This indicates that not only can the gelatin leave the blood stream but it can be excreted by the kidneys as well.

In nephritis. There is also considerable difference in the relative ease with which the nephritic rabbits disposed of the colloidal injections. Experiment 52 shows a case where the colloidal solution left the circulation with no greater difficulty than a saline solution. In experiment 56, however, a large proportion of the solution was unable to leave the blood vessels during the experiment. The amount of the injected fluid still in the circulation after one hour varied from 11 to 57 per cent.

Experiment 52. Rabbit ♀, nephritic weighing 2600 grams.

October 15—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

October 19—Urine for previous twenty-four hours, 6 cc., which contains sugar and protein. Animal in good condition.

Injection of 125 cc. of gelatin solution in 3 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	43	100
After injection		
Immediately	34	126
5 minutes	35	122
10 minutes	36	119
15 minutes	38	113
20 minutes	38	113
25 minutes	36	119
30 minutes	38	113
35 minutes	38	113
40 minutes	38	113
45 minutes	39	110
50 minutes	38	113
60 minutes	38	113

Kidneys were very pale, with marked edema; bladder was empty; pleural and peritoneal cavities each contained 2 to 3 cc. of clear fluid.

Experiment 56. Rabbit ♂, nephritic, weighing 1980 grams.
October 19—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.
October 23—Animal used for injection of gelatin solution.
Injection of 98 cc. of gelatin solution in 2 minutes 25 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	51	100
After injection		
Immediately	30	170
5 minutes	34	150
10 minutes	35	146
15 minutes	35	146
20 minutes	34	150
25 minutes	35	146
30 minutes	35	146
35 minutes	35	146
40 minutes	35	146
45 minutes	35	146
50 minutes	35	146
60 minutes	35	146
70 minutes	35	146

Kidneys were pale and enlarged, with marked edema; in the bladder were 43 cc. of light, turbid urine, containing a small amount of sugar and coagulable protein and giving a strong biuret test; there were 4 cc. of clear fluid in the peritoneal cavity while the pleural cavity contained no fluid.

The average figures obtained from the experiments with injections of colloidal solution in normal and nephritic rabbits are given in table 9 and these data were used in plotting the curves in the accompanying chart.

TABLE 9

Regulation of the blood volume after injections of saline-colloidal solution

Average figures

	RELATIVE BLOOD VOLUMES AFTER												
	Injection	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min.	60 min.	70 min.
Normal rabbits—Group I	173	134	123	117	117	117	111	107	103	103	102	100	100
Normal rabbits—Group II	170	157	146	142	130	127	125	124	122	120	121	117	118
Nephritic rabbits	152	143	138	136	136	136	135	135	134	133	134	135	

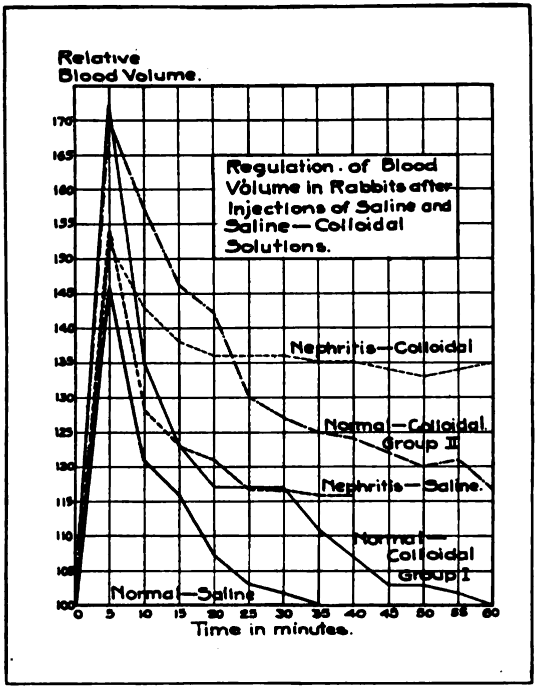

Fig. 1

EXPERIMENTS WITH DOGS

Under normal conditions and in nephritis. Both the normal and nephritic dogs disposed of the gelatin injections with ease. As morphine-atropine injections were given in each experiment, the animals were never able to completely restore their blood volume to normal but they did so to as great or greater extent than after saline injections. The colloidal injections showed a marked lymphagogic action. The average amount of the injected fluid collected as lymph is 25 per cent in the normal dogs and 34 per cent in the nephritic dogs. These figures are slightly larger than corresponding figures after saline injections. These animals, therefore, had no difficulty in expelling from the circulation excess of fluid held in colloidal solution. The protocols which follow show the course of two such experiments and the general results are shown in the accompanying charts.

Experiment 34. Dog ♂, normal, weighing 13 kg.

8.30 a.m.—Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

9.30 a.m.—Anaesthetized with ether. Cannula in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE BLOOD VOLUME
	Time	Interval flow	Time	Hemoglobin	
		cc.		per cent	
	10.03		10.03	80	100
10.10–10.12 Injection I of 325	10.08	1.7			
cc. gelatin solution	10.13	1.2	10.12	60	133
	10.18	7.9	10.17	65	123
	10.23	10.1	10.22	67	118
	10.28	8.5	10.27	69	116
	10.33	6.5	10.32	72	111
	10.38	5.5	10.37	72	111
	10.43	5.0	10.42	71	113
10.50–10.52 Injection II of 325	10.48	4.1	10.47	71	113
cc. gelatin solution	10.53	3.6	10.52	57	140
	10.58	17.7	10.57	60	133
	11.03	14.5	11.02	60	133
	11.08	11.5	11.07	62	129
	11.13	10.8	11.12	65	123
	11.18	10.5	11.17	68	118
	11.23	10.2	11.22	68	118
	11.28	10.8	11.27	68	118
	11.33	9.5			
11.39–11.43 Injection III of 650	11.38	10.4			
cc. gelatin solution	11.43	10.0	11.43	50	160
	11.48	38.5	11.48	52	154
	11.53	36.6	11.53	53	151
	11.58	26.0	11.58	56	143
	12.03	23.1	12.03	57	140
	12.08	21.0	12.08	59	135
	12.13	22.6	12.13	58	138
	12.18	18.5	12.18	62	129

There was no fluid in either of the body cavities; in the bladder were 100 cc. of medium colored urine which contained no coagulable protein but gave the biuret test.

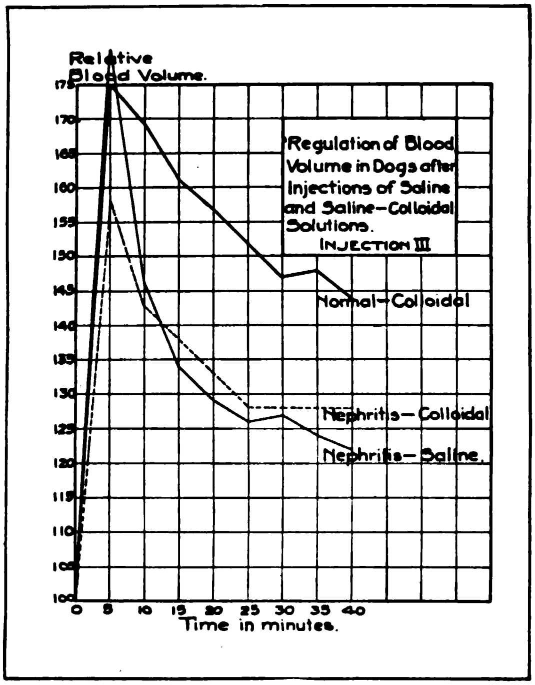

Fig. 4

CONCLUSION

Fluid injected in the form of colloidal solution *may* leave the circulation as readily as that in non-colloidal solution; but *usually the regulation of the blood volume is less efficient after the injection of colloidal solutions.*

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THE INFLUENCE OF ALKALINE-SALINE SOLUTIONS UPON REGULATION OF BLOOD VOLUME[1]

STUDIES OF THE PERMEABILITY OF CELLULAR MEMBRANES. III.

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The injection of alkaline solutions having been proposed by Fischer ('15)[2] as a means of dehydrating the colloids of the body, it was thought of interest to determine whether the regulation of the blood volume after injections is any different, especially in nephritic animals, when alkali is added to the injection fluid.

Sodium carbonate was dissolved in 0.9 per cent sodium chloride solution, as Ringer's solution could not be used on account of the precipitation of the calcium as calcium carbonate. Solutions equivalent to 0.2 per cent and 0.4 per cent sodium carbonate were injected,[3] the size of the injection being in each case equal to the calculated blood volume of the animal. The effect of such injections is seen in the following specimen protocols.

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[1] The data in this paper are taken from the dissertation presented by L. Jean Bogert for the degree of Doctor of Philosophy, Yale University, 1916.

[2] Fischer, Oedema and Nephritis, Edition 2, John Wiley and Sons, 1915.

[3] For description of the technique of these experiments see this Journal, 1916, xli, 189.

Experiment 41. Rabbit ♀, normal, weighing 3360 grams. Injection, in 3 minutes 25 seconds, of 165 cc. of *0.2 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HÆMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	51	100
After injection		
Immediately	36	141
5 minutes	43	119
10 minutes	44	115
15 minutes	47	108
20 minutes	49	104
25 minutes	51	100
30 minutes	51	100

Bladder contained 38 cc. of urine, secreted during the experiment, which gave a very faint test for protein; animal was pregnant; there were 6 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

Experiment 42. Rabbit ♀, normal, weighing 1680 grams. Injection, in 3 minutes 15 seconds, of 85 cc. of *0.4 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HÆMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	57	100
After injection		
Immediately	35	163
5 minutes	44	129
10 minutes	49	116
15 minutes	51	112
20 minutes	54	105
25 minutes	57	100
30 minutes	57	100

Bladder contained 17 cc. of urine, secreted during the experiment, which gave tests for protein; there were 2 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

Experiment 45. Rabbit ♂, nephritic, weighing 1800 grams.

August 18—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

August 19—Urine, 145 cc.

August 20—Urine, 50 cc., thick and turbid.

August 21—Urine, 27 cc., strong tests for both protein and sugar.

Injection, in 4 minutes, of 90 cc. of *0.4 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	41	100
After injection		
Immediately	24	171
5 minutes	31	132
10 minutes	31	132
20 minutes	31	132
25 minutes	32	128
30 minutes	32	128

Bladder was empty; kidneys were very pale and inflamed but with no edema; the pleural and peritoneal cavities each contained 1 to 3 cc. of fluid.

Experiment 49. Rabbit ♀, nephritic, weighing 2600 grams.

October 11—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

October 15—Urine, 10 cc. Sugar and protein both present. Animal lively and in excellent condition. Weight, 2540 grams.

Injection, in 2 minutes 5 seconds, of 125 cc. of *0.4 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	53	100
After injection		
Immediately	34	156
5 minutes	41	129
10 minutes	42	126
15 minutes	45	117
20 minutes	45	117
25 minutes	45	117
30 minutes	45	117

Bladder was empty; kidneys were pale and inflamed; there were 7 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

TABLE 10

Regulation of blood volume after injections of saline and alkaline-saline solutions

Average figures

	RELATIVE BLOOD VOLUMES AFTER						
	Injection	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
Normal rabbits							
Saline injection	146	121	116	107	103	102	100
Alkaline-saline injection	152	119	111	107	104	101	100
Nephritic rabbits							
Saline injection	154	128	123	121	117	117	116
Alkaline-saline injection	171	133	132	123	123	122	122

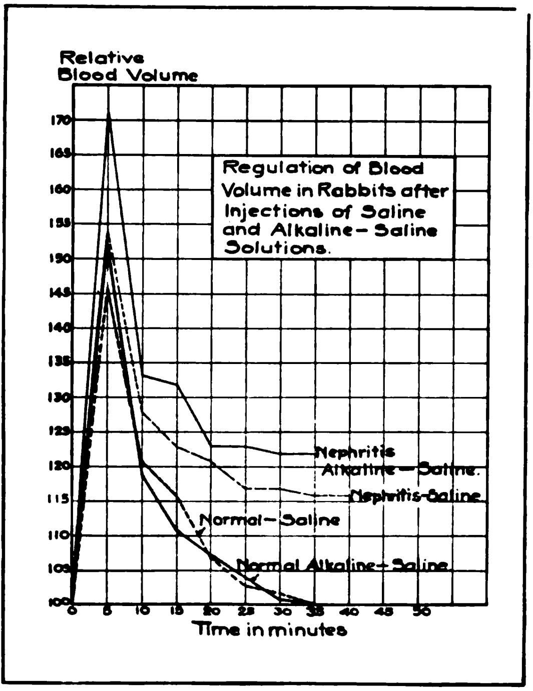

Fig. 1

CONCLUSION

It will be seen from these experiments and the accompanying chart, that *the addition of alkali*, up to 0.4 per cent sodium carbonate, to the fluid injected, *has no apparent effect on the regulation of the blood volume* either in normal or in nephritic animals. The rate at which, and the extent to which, injected fluid leaves the blood vessels is unaffected by the addition of sodium carbonate.

THE INFLUENCE OF IODIN AND SODIUM IODID ON THE CIRCULATION

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Iodin and its compounds have long been recognized among the best remedies in the possession of the physician. Their pharmacological action, however, is still imperfectly understood. This seems to be particularly true of their effect on the circulation, as shown by Capps (1), in a review of the literature on the subject. Some observers seem to believe that they are circulatory depressants while others brought forward evidence indicating that iodin and sodium iodid were, on the contrary, circulatory stimulants. According to Prevost and Binet (2) a fall of blood pressure is caused after intravenous injections into animals, but their effect is different when given by mouth. They maintained that iodin caused a rise of blood pressure, while the iodid failed to produce any effect on the circulation when they were administered by mouth.

Lehndorff (3) stated that sodium iodid injected intravenously into cats was followed by a rise of blood pressure amounting to 50 per cent, which he claimed was due to the increased cardiac output he observed as moderate dilatation of the peripheral arteries occurred at the same time. Macht (4) studied the action of iodin and iodid on the isolated heart of frogs and mammals as well as on intact animals and concluded that both of these substances are cardiac stimulants. His tracings show only a moderate increase in amplitude, but there was pronounced augmentation in the frequency of heart action. He also reported that sodium iodid caused moderate stimulation of the circulation in intact animals, but he failed to obtain any action with iodin.

That the behavior of sodium iodid varies with the condition of the circulation was maintained in a recent communication by Retzlauf (5). He injected intravenously a hypertonic solution of sodium iodid into rabbits whose blood pressure had been lowered by hemorrhage. The first dose showed a marked but transient rise of blood pressure. The effect of successive doses given later diminished with the progressive rise of blood pressure.

In the course of studies on the action of various organic derivatives of iodin, we noticed that they often caused a marked fall of blood pressure. This suggested to us the advisability of testing the effect of molecular iodin and of sodium iodid on the circulation. The results form the subject of the present communication.

Iodin was dissolved in alcohol and was added to Ringer's solution, the alcohol being employed in order to facilitate the solution of iodin. The concentration of alcohol in the perfusion fluid varied between 0.03 and 0.16 per cent. It might be stated in advance, that this had no effect on cardiac action. Iodin was used in the proportions of 1: 30,000; 1 :15,000; 1 :10,000; and 1:5,000, these solutions being perfused for variable periods of time. In some experiments small quantities of olive oil were added to the Ringer-iodin solution. Studies were also made with different concentrations of sodium iodid.

The experiments were conducted on the isolated frog heart suspended in a moist chamber. The perfusing fluids were supplied by reservoirs in which a constant pressure was maintained. By means of rubber tubes these were connected with a four-way stopcock communicating with a cannula in the venous sinus. The heart could then be perfused with any of the fluids contained in the reservoirs. For a detailed description of the apparatus, the reader is referred to a recent publication from this laboratory (6). Experiments with iodin and sodium iodid were also carried out on intact animals, alcohol—chloretone or ether anesthesia being used.

The results of our observations with various concentrations of iodin indicate that iodin may produce disturbance of cardiac activity. Heart action became irregular, relaxation was incomplete, rate and amplitude varied. Periods of normal action alternating with these changes were sometimes also observed. With stronger solutions, complete suspension of activity occurred and lasted one-half to one minute. In nearly every case, depressing after effects which occasionally became very pronounced, were also observed. Some of these changes could be induced with dilute concentrations of iodin even in the fresh heart, which was found to be more resistant to poisons than at later stages of the experiment.

EXPERIMENTS WITH 1:30,000 IODIN

The results obtained indicated that a concentration of 1:30,000 iodin may cause disturbance of heart action, but the effect was never very pronounced and varied considerably. Thus, in one experiment in which perfusion with iodin lasted two minutes, a decrease of amplitude with a corresponding increase in rate were observed within one-half minute after the iodin solution began to enter the heart. The action of iodin in the other experiments showed a tendency to cause diminished activity which was sometimes preceded by stimulation. Irregular heart action was the main effect produced in other experiments which appeared within three-fourths to two and one-fourth minutes after perfusion was begun. Depressing after effects were frequently observed (figs. 1 and 2).

EXPERIMENTS WITH 1:15,000 IODIN

The action of iodin in these experiments likewise varied. Iodin in Ringer's solution and 0.06 per cent alcohol caused in some cases a decrease in rhythm, amplitude being slightly increased at the same time. Irregular heart action, as observed with more dilute solutions of iodin, was also present, but was more pronounced. This is shown in figures 3 and 4 and in the following experiments which are typical instances of the behavior of the heart when subjected to the action of a solution of iodin 1:15,000.

Frog 145 B. IODIN 1:15,000 PERFUSED FOR THREE MINUTES

About forty-five seconds after perfusion was begun, the frequency of the heart diminished for about one-half minute, while the force of the contractions became appreciably greater, a tendency to incomplete relaxation being observed, however, at the same time as after every seventh or eighth contraction the diastole was about a quarter in extent of the one preceding or following it. Toward the end of the perfusion, every five or seven contractions were separated by a considerable interval of rest, the contractions in the interim being regular, but the amplitude steadily decreased. One and a half minutes after perfusion with iodin, the heart began to grow weaker until the contractions were hardly perceptible four minutes later. The action of iodin when used in this concentration was sometimes even more pronounced, as was shown in the following case.

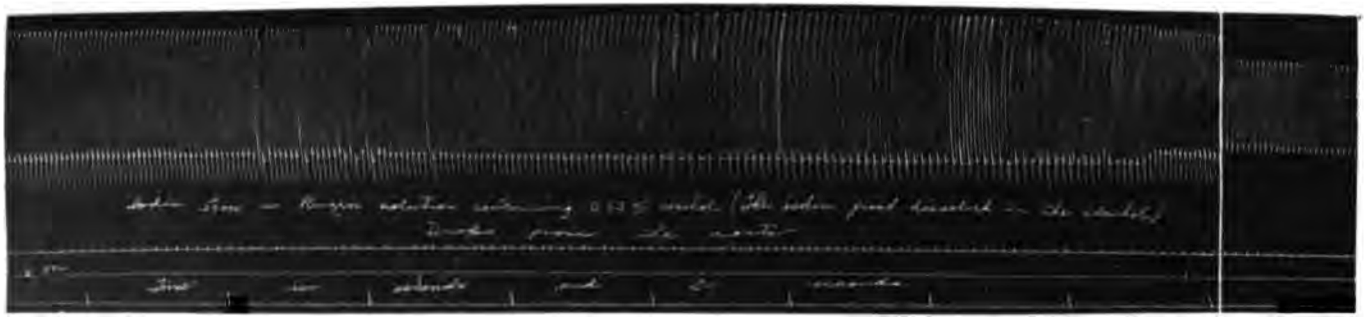

Fig. 1. Frog 145. *A*, heart action became irregular as a result of perfusion with iodin 1: 30,000. *B*, cardiac depression which commenced in the after period reached a maximum six minutes after perfusion with iodin was discontinued

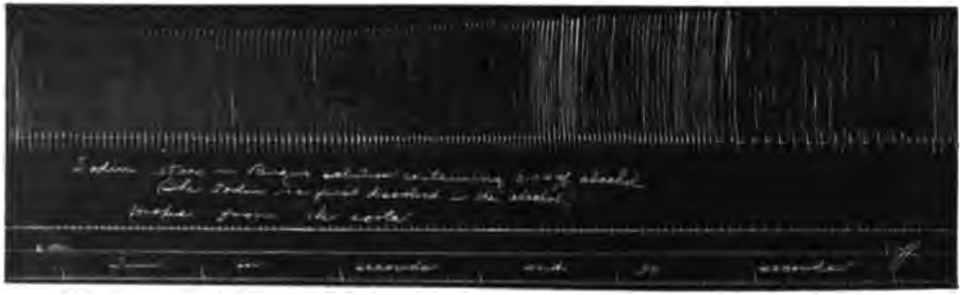

Fig 2. Frog 144. Perfusion the same as in figure 1. Initial stimulation is followed by cardiac irregularity

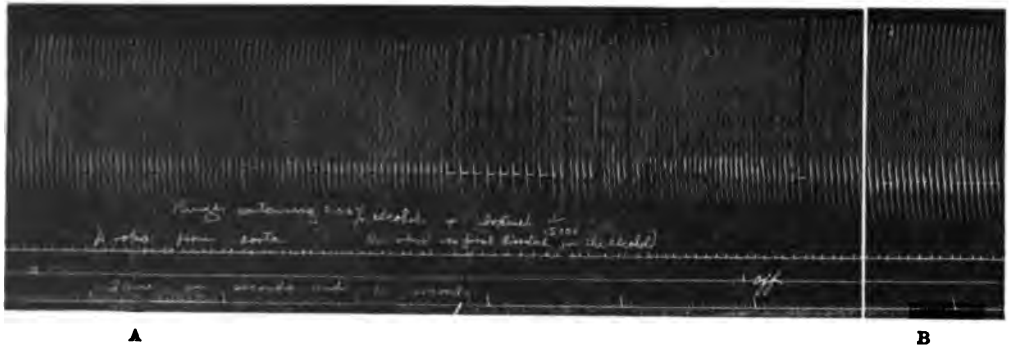

Fig. 3. Frog 156. *A*, iodin causes decrease of frequency at first then irregular action and stimulation. *B*, four to five minutes after iodin was perfused.

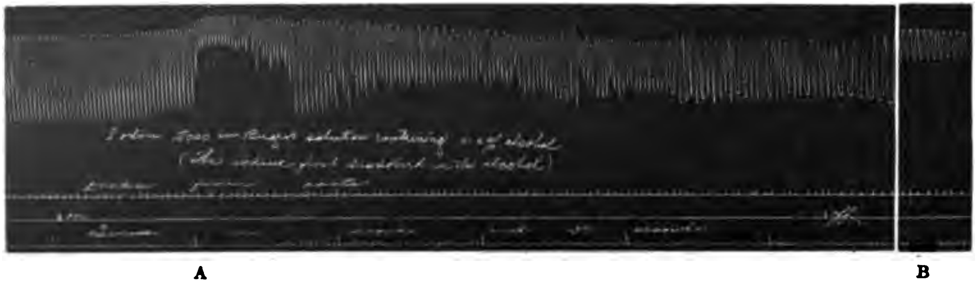

Fig. 4. Frog 143. *A*, marked irregularity as a result of perfusion with 1: 1,500 iodin in alcoholic-Ringer solution. *B*, two minutes after iodin. Shows depression in the after period.

Frog 143. After exposure for forty-five seconds only, cardiac activity was reduced, contractions became both feeble and irregular. Recovery was prompt, however, when iodin was discontinued. After a second perfusion for two and one-half minutes, made ten minutes later, the effect was very marked. Examination of figure 4 will show that heart action was reduced about one-third within half a minute and became irregular during the rest of the perfusion. Pronounced depression was observed after iodin was discontinued.

Experiments were also carried out in which small quantities of olive oil were added to the Ringer-iodin solution. The injury caused by iodin was much less marked in these cases. Thus, although depression was observed in one experiment in which the duration of the perfusion was six minutes and forty seconds, no effect could be noticed during the first three minutes. In another experiment in which perfusion with iodin lasted four minutes, depression appeared one and one-half minutes after the solution entered the heart. That olive oil and iodin are antagonistic was shown very strikingly in several cases in which the perfusion periods were much shorter. Thus in three tests performed on two specimens, two, three and one-fourth and three minutes were allowed for perfusion with iodin and olive oil. Little or no depression occurred during the exposure to this combination nor were after effects observed. There was on the contrary distinct stimulation in one case (fig. 5).

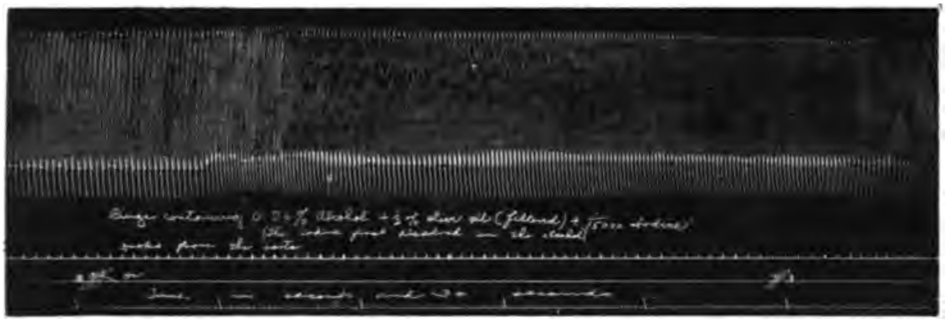

Fig. 5. Frog 149. Effect of 1: 15,000 iodin in Ringer-olive oil and alcohol. Note absence of irregularity and compare with figure 4.

EXPERIMENTS WITH 1:10,000 IODIN

Within one-half to one and one-half minutes after the heart was subjected to the action of iodin and 0.08 per cent alcoholic Ringer's solution, marked disturbance of cardiac action appeared. Force and frequency varied under its influence. Systole was either moderately increased or diminished, diastole was frequently incomplete, all these changes being sometimes observed in the same experiment. In some instances, however, the action of iodin was not very marked. When perfused for two or two and one-half minutes, irregularity of action lasting about one-half minute was noticed in two cases toward the end of the experiment, a decrease of amplitude and increase of rate being observed in a third experiment after one and one-fourth minutes, while moderate increase in the force of contraction was noticed in a fourth case after the same interval of time. Depressing after effects, as already noted with more dilute solution, were more frequent when the concentration of iodin was 1:10,000. The more typical action of the heart when perfused with such a solution is shown in the following experiments:

Frog 144. Perfused three minutes with 1:10,000 iodin in 0.08 per cent alcoholic Ringer's solution. Within less than half a minute heart action became irregular, periods of incomplete relaxation alternating with those of increased frequency. For two and one-half minutes after perfusion with iodin, heart action was normal, but a gradual decrease in the force of contraction set in now. Four and a half minutes later the amplitude was decreased about 70 per cent.

Frog 141. Perfused six minutes with iodin in 0.08 per cent alcoholic Ringer's solution. Within one and one-half minutes after the experiment with iodin was begun, heart became irregular, frequency fluctuated sometimes being greater, sometimes less, than before iodin. Incomplete relaxation also occurred at irregular intervals. A short period of improvement followed after iodin was discontinued, but force and frequency soon began to diminish, heart action becoming very weak in two and one-half minutes.

Frog 146. Perfused with 1:10,000 iodin in 0.08 per cent alcoholic Ringer's solution. About half a minute after the experiment with iodin was begun, irregularity of heart action preceded by a brief period of increased frequency, was observed. Within a half minute, heart action became regular once more, force and frequency being moderately increased at the same time This lasted one and one-fourth minutes

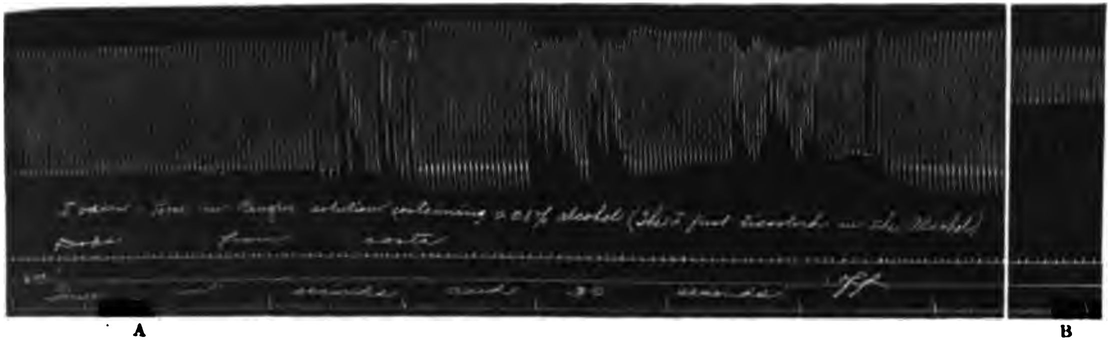

Fig. 6. Frog 144. Perfusion with 1: 10,000 iodin in Ringer's solution. *A*, heart action became irregular about one minute after perfusion with iodin was begun. Note intervals of improvement. *B*, cardiac depression set in sometime after perfussion with iodin was stopped and continued steadily until it reached a maximum as shown in figure.

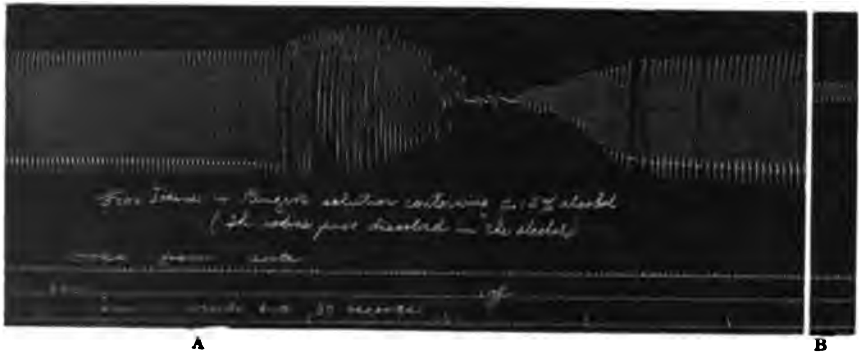

Fig. 7. Frog 139. *A*, 1: 5,000 iodin in Ringer-alcoholic solution produced cardiac irregularity and arrest of heart in diastole; also shows recovery when iodin perfusion was discontinued. *B*, depression in after period five minutes after iodin.

when heart action became irregular again, groups of contractions separated by considerable intervals of rest being observed. The perfusion lasted four minutes. The usual depressing after effect was observed. Force of contractions began to diminish steadily after iodin perfusion.

Frog 135. Perfused two minutes with iodin in alcoholic Ringer's solution. Within one and one-half minutes after the experiment was begun the amplitude increased, but the contractions were less frequent. This was followed by a period in which the frequency and force of the contractions exhibited considerable variation. The heart was under observation three and three-fourths minutes after iodin was discontinued, but no depressing after effects were noticed. Marked effects were also observed in another test in this experiment in which perfusion with 1:10,000 in alcoholic Ringer's solution was carried out for one minute only. After a period of depression lasting two minutes, during which heart action was abolished for a half minute, recovery set in.

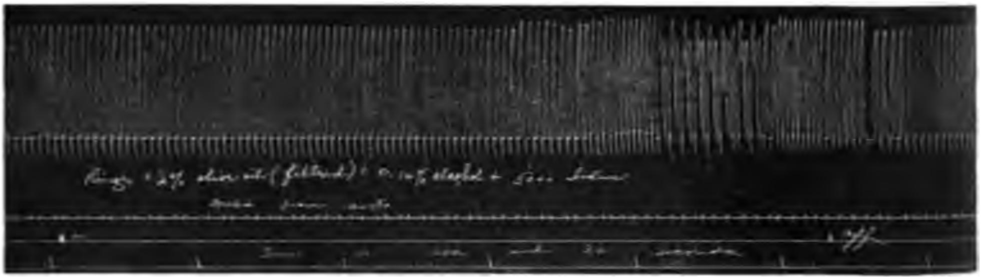

Fig. 8. Frog 153. Iodin 1:5,000 in alcoholic-Ringer's and olive oil. Irregular heart action of short duration. Compare figure 7.

EXPERIMENTS WITH 1:5,000 IODIN

Two series of experiments were carried out in one of which iodin was dissolved in alcoholic Ringer's solution while the other also contained olive oil. The behavior of the heart in the two cases was noticeably different. After one to two minutes perfusion with the iodin solution without olive oil, arrest of the heart in diastole was observed in two cases and a very marked decrease in the contractions occurred in the third. The other effects such as irregular heart action during perfusion and depression when iodin was discontinued were also present, and were much more pronounced than in experiments in which weaker solutions were used. In similar tests with iodin and olive oil, the results were not quite the same. No change for two minutes could be noticed in one case, the heart suddenly became irregular, but recovery followed in less than half a minute. Marked effects were noticed in experiments on two other specimens perfused for one and one-half minutes and four and one-half minutes, but the heart never came to a standstill.

EXPERIMENTS WITH SODIUM IODID

Perfusion of the isolated frog heart with Ringer's solution to which 0.5, 1 and 2 per cent of sodium iodid were added caused depression which increased with the concentration of the iodid. This was due, however, to the increased osmotic pressure as shown in control tests with Ringer's solution to which addition of equivalent amounts of sodium chlorid produced similar effects. The action of sodium iodid was also tested by means of Ringer's solution in which the sodium chlorid was either partly or entirely replaced by equivalent amounts of sodium iodid, the composition of the solutions being: NaCl 2.1; KCL 0.075; $CaCl_2$ 0.1; $NaHCO_3$ 0.05; NaI 10.0 gms. or NaCl was entirely replaced by 15.4 gms. NaI per liter. Perfusion for one to six minutes with either of these solutions produced depression of amplitude varying in degree, rate being seldom affected (see figs. 9, 10). Recovery was almost always observed when perfusion with iodid was discontinued. In a smaller number of cases, however, sodium iodid was without any effect.

The condition of the heart seemed to be an important factor in determining the action of iodid since we often noticed marked differences in the reaction of the fresh heart and the heart that had been perfused for some time and thus approached a hypodynamic condition. As shown in figure 9, sodium iodid in Ringer's solution perfused soon after the heart was isolated was without any effect but the same treatment one hour and thirty-seven minutes later caused marked depression. The reaction of the heart to sodium iodid which was added to Locke's solution was also tested in a number of experiments. Macht stated that sodium iodid stimulated heart action, force and frequency being increased according to his records. We perfused the heart with a solution made up as follows: NaCl 4.5; KCl 0.42; $CaCl_2$ 0.24; $NaHCO_3$ 0.15; dextrose 1.0; NaI 11.5, gms. per liter, employed by Macht, or NaCl 3.0 and NaI 15.4 grams per liter, all the other ingredients being the same in both solutions. The results obtained varied con-

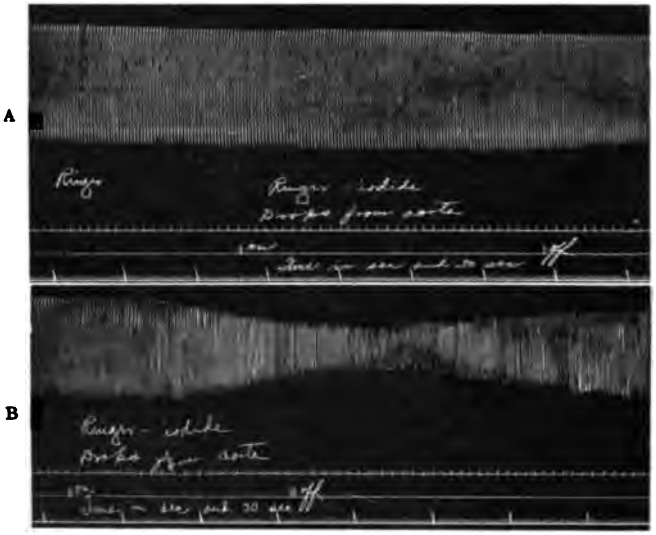

Fig. 9. Frog 189. *A*, perfusion of the fresh heart with Ringer iodid. *B*. perfusion with Ringer iodid one hour and twenty-two minutes after *A*.

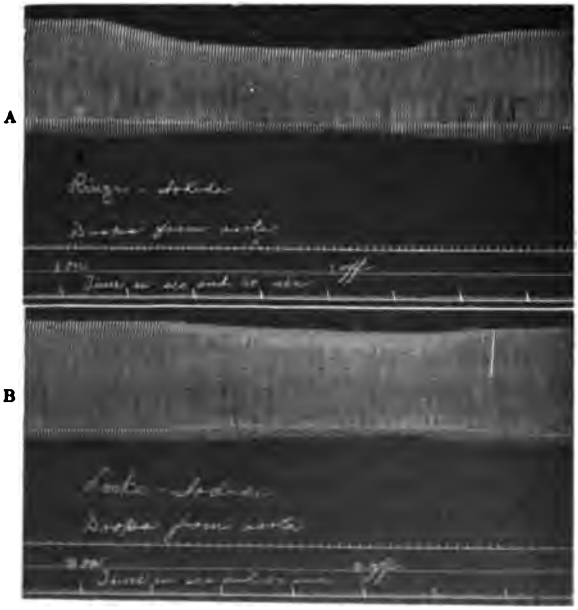

Fig. 10. Frog 197. Shows the reaction of the same heart to (*A*,) Ringer and (*B*) Locke solutions in which the same amounts of sodium iodid (15.4 grams per liter) were substituted for equivalent amounts of sodium chlorid.

siderably. Depression of amplitude was observed in some experiments (figs. 10B and 12B), in others frequency was markedly increased (fig. 12A), a moderate decrease of amplitude being present at the same time. In a third group of cases, force as well as frequency were increased (fig. 11, *A*, *B*). Occasionally the heart almost failed to react to the solution (fig. 11, *C*). The greater resistance of the fresh heart was also frequently noticed in experiments with solutions of Locke-iodide as shown in some of the figures to which reference has already been made.

DISCUSSION

The striking difference in the action of iodine and sodium iodid observed in these experiments are in harmony with the observations of previous investigators on the toxicity of these substances. The experiments of Overton (7) and those of Stockman and Charteris (8) have shown that molecular iodin is much more toxic than its sodium salt. More recently, Adler (9) studied the influence of sodium iodid and of various organic preparations on the sexual organs of the rabbit. His observations show that the alkali iodides are less injurious than organic iodin preparations.

An explanation of the action of iodin compounds had first been sought by Oswald Loeb (10). On the basis of his experiments with potassium iodid and a number of organic preparations, he formulated the lipotropic theory according to which the action of iodides depends on their solubility in the lipoids of the tissues. Support was given to it later by the work of McLean (11). He found that potassium iodid and various organic preparations when brought in contact with liver tissue *in vitro* or when administered to rabbits, are absorbed by the tissue lipoids. This would not explain, however, the reaction of the heart to iodin and sodium iodid which was observed in the present investigations, nor the enormous difference in the toxicity which has been demonstrated by several other observers. In this connection the investigations on tissue lipoids made within recent years are particularly interesting. Erlandsen (12) isolated from the heart, a substance which he called cuorin, containing acids of the linolenic and linoleic groups with an iodin number of 130.1. According to Leathes (13) the lipins of the heart contain acids which are highly unsaturated. The formation of addition products with molecular iodin may therefore be expected. The more stable product thus formed would become less readily available for cardiac metabolism since, as pointed out by Dakin (14), the saturated fatty acids are not oxidized as readily as the unsaturated compounds. Indeed it is conceivable that the oleic acid radicle may become transformed into a more stable form with a much higher melting point and the resulting product may even become injurious. The failure to react to iodin injected into dogs intravenously may be explained by the formation of a similar combination with the lipins of the blood, the case being analagous to the effect of iodin when olive oil was added to Ringer's solution. But this would seem to be contradicted by the results obtained in cats. It may be recalled that according to Abderhalden (15), the lipin content in the blood serum of these animals exclusive of cholesterin is about half that in the serum

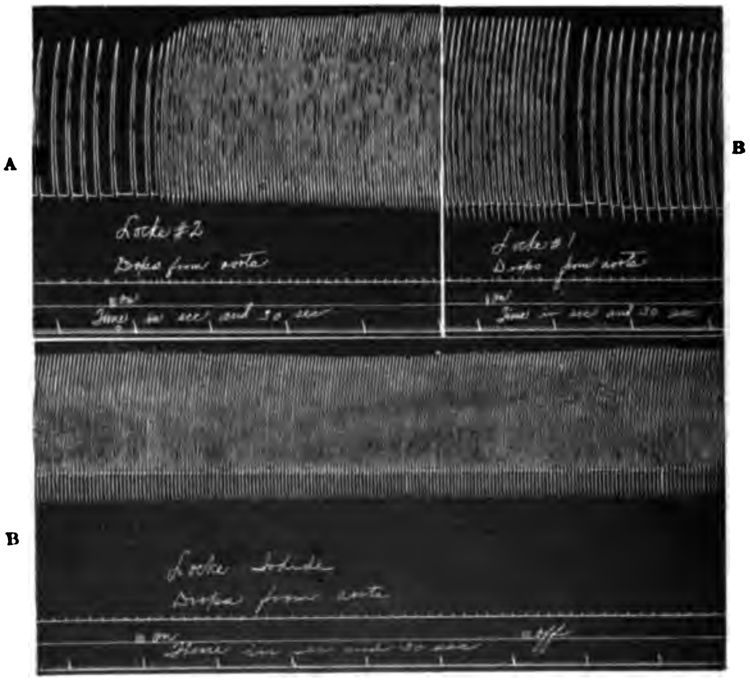

Fig. 11. Frog 185. *A*, shows stimulating effect of Locke iodid (Locke No. 2, 4.5 grams NaCl per liter replaced by equivalent amount of sodium iodid). *B*, condition of heart after seven minutes perfusion with Locke iodid and recovery when Locke No. 1 (Locke solution without iodid) was perfused. *C*, frog 198, shows only slight depression caused by Locke iodid solution (Locke solution in which 6 grams sodium chloride per liter was replaced by an equivalent amount of sodium iodid).

of the dog's blood. Moreover, the heart being more active, probably contains acids that are more unsaturated than those in the dog heart. According to Leathes (16), the more unsaturated fatty acids are found in locations of more active metabolism. Accordingly the fat of the heart would absorb more iodin than that of the connective tissue. Hence, the more rapid the cardiac action, the more unsaturated the fats contained in the heart. Thus the iodin number of the fat obtained from the dog's heart is 70, from that of the sheep 58.

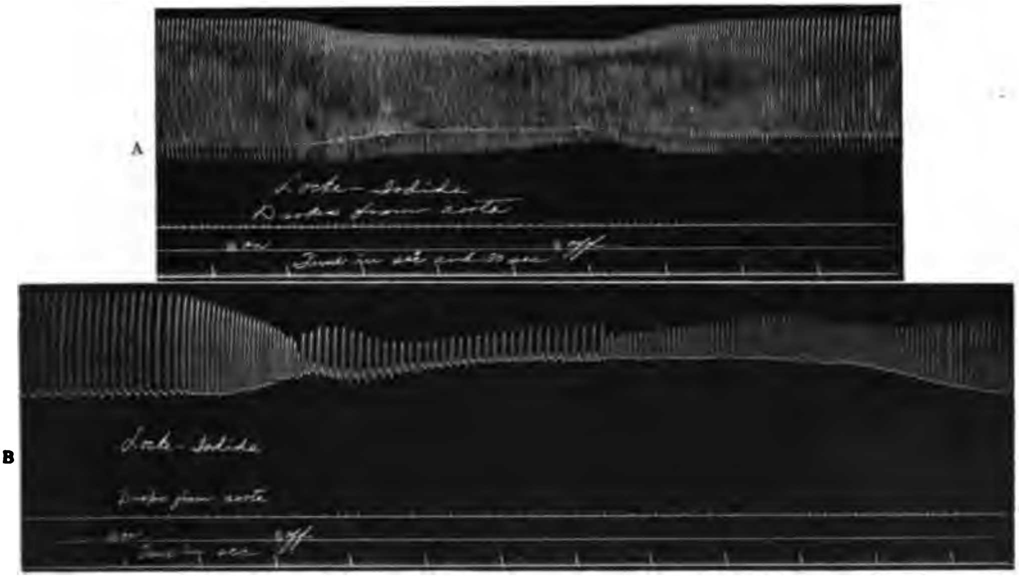

Fig. 12. Frog 191. Note reaction of heart to Locke iodid (Locke solution in which 15.4 grams NaI per liter replaced equivalent amounts of sodium chlorid.) *A*, at the beginning of the experiment. *B*, one hour and fifteen minutes after *A*.

The action of sodium iodid on the frog heart is more difficult to explain. Iodin in the ionic state does not enter into combination with the unsaturated fatty acids in a manner analagous to free iodin. There is evidence, however, that iodin is taken up from potassium iodid by the tissue lipoids. Although the experiments of Oswald Loeb (17) failed to show its presence in brain, spinal cord, and tissue fat after the administration of potassium iodid, later investigations revealed its presence in the lipoids of various organs after treatment with inorganic iodids. Thus Boruttau (18) found iodin in the brain and McLean (19) found it in the lipoids of the liver after giving potassium iodid or after adding it to the liver *in vitro.* The mechanism in this case is probably due to physical action between the lipoids and ionic iodin.

The failure to produce any effect on the circulation after the introduction of large amounts of sodium iodid may be accounted for in part by rapid elimination, but it is also probable that it is bound in some way by the lipoids of the blood, thus preventing its action on the heart and blood vessels.

SUMMARY AND CONCLUSIONS

Dilute solutions of molecular iodin perfused through the isolated frog heart produce cardiac irregularity and depressing after effects. After more concentrated solutions, heart action becomes much more irregular and the heart may be arrested in diastole. The after effects are also more pronounced. The presence of olive oil in the perfusion fluid decreases the toxicity of iodin. Marked depression of the circulation was observed in cats after intravenous injection of iodin, but no change could be noticed in dogs similarly treated. Sodium iodid is much less toxic than iodin. A solution of ½ per cent sodium iodid added to Ringer's solution perfused through the frog heart produced slight depression. In no case was an injurious after effect noted. Sodium iodid injected intravenously into cats and dogs failed to produce any effect on the circulation even when moderately large doses were introduced rapidly.

It is suggested that the action of molecular iodin is due to formation of additive compounds with the unsaturated fatty acids present in the frog heart. The action of iodin on the circulation of the cat might be due to the presence in the heart of large amounts of unsaturated fatty acids or perhaps to acids that are more unsaturated than those in the heart of the dog and also to the presence of smaller amounts of some of the lipoids in the blood serum of the cat than in that of the dog.

The action of sodium iodid on the frog heart is probably due to physical action with the lipoids of the heart.

Rapid elimination was suggested as a possible cause of the negative results obtained after the intravenous administration of sodium iodid to cats and dogs.

Ringer's solution in which the sodium chlorid was replaced by equivalent amounts of sodium iodid generally produced cardiac depression. The results with Locke's solution containing the same amounts of sodium iodid were not constant, sometimes stimulation and sometimes depression being observed, while in some cases no effect on the heart could be noticed as a result of the same treatment. 0.03 to 0.16 per cent alcohol in Ringer's solution had no effect on the frog heart.

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THE THROMBOPLASTIC ACTION OF CEPHALIN

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In 1912 Howell (1) reported the results of a study of the thromboplastic action of the tissues in which he showed that the active substance is a phosphatid having the general properties of cephalin. The effect of solutions of this phosphatid upon coagulation is very striking and it has been shown that lecithin prepared according to the customary methods is lacking in this property. The identification of the phosphatid rested mainly on its insolubility in cold alcohol and its slight solubility in hot alcohol and the possibility was recognized that the thromboplastic action might be due to some adherent impurity rather than to the phosphatid itself. At the suggestion of Dr. Howell I have undertaken a re-examination of this subject to determine if possible whether the thromboplastic effect may be attributed to an impurity, or is a property of the cephalin itself, and also to determine in how far a similar property is exhibited by other related phosphatids. The phosphatids which have been examined in regard to their thromboplastic action are cephalin, lecithin, sphingomyelin, cuorin and heparphosphatid.

THE METHOD OF TESTING THROMBOPLASTIC ACTIVITY

To determine thromboplastic activity a method was employed which has been in use in this laboratory for some time in connection with other researches on coagulation. The method rests upon the fact that in fresh serum the amount of actual or effective thrombin is small, but the amount of ineffective thrombin is relatively large. This metathrombin may be converted to active thrombin by Morawitz's (2) method of adding alkali with subsequent neutralization. But it can also be activated to effective thrombin by the addition of thromboplastic extracts, especially solutions of cephalin, provided the serum is perfectly fresh. As the serum stands the activating effect of the cephalin becomes less marked and finally disappears entirely after a certain variable time. Long after cephalin activation has ceased entirely the alkali activation is still effective. This activating effect of cephalin on fresh serum was used in the following way. Fresh serum and fresh oxalated and centrifugalized plasma were prepared from the same animal. By trial the amount of serum was determined which would cause clotting of a definite amount of plasma within a time convenient for observation—say thirty minutes to two hours. The thromboplastic activity of cephalin or any other solution under observation was determined by adding it to the serum and noting the acceleration in the clotting time. Ordinarily the serum of dogs and cats was used and the proportions taken were two or three drops of serum to eight drops of plasma. With these proportions clotting occurs usually in from 30 minutes to 2 hours, but if to the mixture one adds a solution of cephalin or a saline extract of fresh tissue clotting occurs promptly in from 30 seconds to 2 or 3 minutes. The tests were carried out according to the following example:

Control—Oxalated plasma, 8 drops; serum, 3 drops; water, 3 drops. Clot forms in from 30 minutes to 2 hours and usually the clot is imperfect.

Solution to be tested; e.g., solution of cephalin—Oxalated plasma, 8 drops; serum, 3 drops; cephalin solution, 3 drops. A solid clot forms in one minute plus or minus.

In all of the experiments the solutions of the purified phosphatids were compared not only with a control of plasma, serum and water, but also with a control made up with an incompletely purified but very active preparation of cephalin made from the pig's brain. This form of cephalin is now used constantly in the laboratory for experimental and therapeutical purposes and for brevity's sake is designated in the following experiments as "laboratory cephalin." Its mode of preparation is as follows:

PREPARATION OF LABORATORY CEPHALIN

A fresh brain obtained from the slaughter house is freed as far as possible from membranes and blood and is then ground to a pulp in a mortar.. The pulp is spread as thin as possible on a glass plate and dried in a current of warm air. When thoroughly dry the material is ground to a powder and is extracted for 4 or 5 hours with an excess of ether. The ether solution is filtered off in a closed space and is passed through the filter paper two or more times until the filtrate comes through clear. The ether filtrate is allowed to evaporate in a current of cool air to a moist residue. The residue is extracted twice with an excess of acetone for 15 to 20 minutes and then twice with an excess of 95 per cent alcohol. Finally the residue is treated again with acetone, the acetone drained off, and the material dried in a desiccator. In using the cephalin for thromboplastic experiments a 0.1 per cent solution is made up in distilled water.

CEPHALIN PREPARED BY THE METHOD OF RENALL

Cephalin was prepared according to the modification of Parnas's (3) method proposed by Renall (4), and by which he states that a yield of very pure cephalin may be obtained. Fresh pig's brains obtained from a slaughter house are carefully freed from membranes and blood; then macerated in a mortar to a fine pulp. This pulp is placed directly into three times its volume of acetone for a day, with occasional shaking. The acetone extract is centrifugalized off and the same amount of fresh acetone added to the residue. After remaining a day in this acetone and being subjected to occasional shaking, the insoluble portion is centrifugalized away from the acetone solution. The residue is shaken for an hour with cold absolute alcohol and the insoluble portion, after removal of the alcohol, is treated twice with three times its volume of petroleum ether of low boiling point (used instead of ethyl oxide to prevent a certain amount of oxidation which is liable to occur with the use of ordinary ether). The petroleum ether extracts, which are colorless or of a lemon yellow tinge, are concentrated in an atmosphere of carbon dioxide and precipitated by pouring into absolute alcohol. The cephalin falls as a white flocculent precipitate, which when dry has a white waxy appearance and forms with water an opalescent solution resembling that of laboratory cephalin. It possesses very active thromboplastic power as is shown in the following experiment.

Dog's oxalated plasma and dog's serum

Using—Plasma, 8 drops; cephalin, 2 drops; serum, 2 drops.
Control—Plasma, 8 drops; water, 2 drops; serum, 2 drops.

Renall's cephalin	good clot	1½ min.
Laboratory cephalin	solid clot	1 min.
Control	no clot in	30 min.

To the precipitate obtained above cerebrosides are adherent as impurities. They are removed by redissolving the precipitate in petroleum ether and allowing the solution to stand over night in the cold. The cerebrosides settle out and by centrifuging are separated from the solution. They are washed with ether and dried. When tested in aqueous solution for thromboplastic activity they were found to be wholly negative. The petroleum ether solution is concentrated and poured into absolute alcohol—the cephalin is precipitated as a white amorphous and somewhat waxy mass, which after drying is made up into a thin emulsion with water and precipitated with normal hydrochloric acid. The acid is added to the emulsion until a good cheesy precipitate is obtained, which settles to the bottom of the container. It is collected by centrifugalization, taken up in a little ether and precipitated with acetone and dried. It forms an opalescent solution in water which upon trial shows marked thromboplastic activity as is indicated in the following typical experiments:

Dog's oxalated plasma and serum

Series I. Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Renall's cephalin	good clot	2 min., 25 sec.
Laboratory cephalin	solid clot	2 min., 45 sec.
Control	poor clot	2 hours, 15 min.

Series II. Using—Plasma, 8 drops; cephalin, 2 drops; serum, 2 drops.
Control—Plasma, 8 drops; water, 2 drops; serum, 2 drops.

Renall's cephalin	good clot	3 min.
Laboratory cephalin	solid clot	1 min.
Control	minute clot in 5 min.	not solid in 30 min.

Thus purified cephalin prepared with a view to the prevention of oxidation and to the removal of adherent impurities possesses thromboplastic activity to a marked degree.

CEPHALIN PREPARED BY THE METHOD OF LEVENE AND WEST

Through the kindness of Dr. Levene a specimen of this cephalin was furnished in order to test its thromboplastic action. The mode of preparation of the specimen is described by the authors (5) and it will be remembered that in the quantitative hydrolysis made by them only 90 per cent of the original weight of material was obtained in the hydrolytic products. The authors therefore consider the possibility that the material contained some impurity. The sample furnished had been precipitated by hydrochloric acid from aqueous solution and after treatment with an organic solvent was finally obtained in the form of a dry powder. When this powder was dissolved in water the solution exhibited marked thromboplastic activity as is shown in the following typical experiment.

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Cephalin—Levene and West	solid clot	75 sec.
Laboratory cephalin	solid clot	40 sec.
Control	poor clot	1 hour, 30 sec.

CEPHALIN AND CUORIN PREPARED FROM THE HEART

Erlandsen (6) as a result of an extended research upon the lipoids of the heart came to the conclusion that there is no cephalin in heart muscle, but in its place he discovered a monoamino-diphosphatid related to cephalin which he called cuorin. To determine if this substance had any thromboplastic effect a quantity was prepared for trial according to the following method as given by Erlandsen:

Ox heart is freed from fat, blood vessels, peri- and endocardium and valves, ground in a meat grinder and spread upon glass plates to dry, then ground to a powder and desiccated over sulphuric acid or calcium chloride. The powdered mass is extracted with ether at room temperature, with a frequent change of the ether until all of the ether soluble material is removed. These extracts are united and concentrated to a syrup, then taken up in a little ether and allowed to stand. An insoluble fraction settles out; it is centrifugalized off and discarded. The ether solution is repeatedly precipitated with dry acetone and then with cold absolute alcohol (zero to minus 2°). This last precipitate is a dark brown sticky mass which upon dissolving in ether and precipitating with alcohol is divisible into two substances—one insoluble in alcohol at 60°, the cuorin a light brown powder, and the other appreciably soluble in alcohol at 60°, a substance having the general properties of cephalin. This cephalin was recovered from the united alcoholic extracts, which were concentrated, precipitated with acetone, taken up in a little ether and precipitated by pouring into alcohol to free from lecithin. This precipitate resembles laboratory cephalin, having the same fish-like odor, and is shown to be as active thromboplastically as the laboratory cephalin.

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Heart cephalin	solid clot 37 sec.
Laboratory cephalin	solid clot 37 sec.
Control—not clotted in 1 hour	clotted over night

The cuorin, on the contrary when purified by repeated precipitation in alcohol at 60°, has no thromboplastic effect—indeed it possesses an anticoagulating power as may be illustrated by the following experiment.

Dog's oxalated plasma and dog's serum

Using—Plasma, 8 drops; phosphatid, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Heart cephalin	solid clot 3 min.
Cuorin	not clotted in 6 hours
Control	sliding clot in 9 min.

Cuorin added to blood fresh from the artery will delay its coagulation remarkably. These two phosphatids from the heart have practically the same solubilities, they pass through repeated processes of extraction together and in the final stages are only separated from each other by the solution of the cephalin in alcohol at 60°. It is unlikely that an impurity would adhere to one of these phosphatids and not to the other.

CEPHALIN AND HEPARPHOSPHATID PREPARED FROM THE LIVER

Baskoff (7), in his work on the phosphatids of the liver, undertaken primarily for the study of the jecorin described by Drechsel (8), succeeded in isolating a phosphatid which resembles cuorin, except that analysis shows a N:P ratio of 1:1.5 instead of 1:2. To this phosphatid he gave the name of heparphosphatid. In my experiments the liver was worked up according to Baskoff's method for obtaining heparphosphatid, which is practically the same treatment to which Erlandsen subjects the heart muscle in order to secure his cuorin. The final substance insoluble in cold alcohol was further divided into a fraction insoluble in alcohol at 60° which resembles cuorin and constitutes Baskoff's heparphosphatid, and a portion soluble in alcohol at 60° which is apparently identical with cephalin. When the latter was recovered from the alcoholic solutions as in the case of the cephalin of the heart, it had all of the general properties of cephalin including the power to hasten the coagulation of blood as is shown in this experiment.

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops, water, 3 drops; serum, 3 drops.

Liver cephalin	solid clot 1½ min.
Laboratory cephalin	solid clot 1¼ min.
Control	not clotted in 1 hour and 45 min.

The heparphosphatid on the other hand when purified by many precipitations in alcohol at 60° has no thromboplastic action and in fact shows a marked power to inhibit the coagulation. The anticoagulating action of this phosphatid is being studied and will be reported upon later. Cuorin and heparphosphatid when dry have no odor, but when moist with warm alcohol have a characteristic odor common to both. It is possible that on further purification the heparphosphatid may be shown to be identical with cuorin.

THE ACTION OF SPHINGOMYELIN

Sphingomyelin is assumed to be a diamino-monophosphatid; it is insoluble in ether and alcohol. In the process of extracting organs for phosphatids some may be carried along with the soluble phosphatids but separates out later from the etheral solutions together with their cerebrosides. The impure cerebrosides as previously mentioned were found to possess no thromboplastic action. By the kindness of Dr. Levene a specimen of sphingomyelin was obtained. When tested by the usual method it was found to have no thromboplastic activity.

THE ACTION OF LECITHIN PREPARED BY THE METHOD OF MACLEAN

Howell (1) showed clearly that lecithin as customarily prepared from the brain and from the yolk of egg exhibits no thromboplastic action. At that time there was no satisfactory method for the absolute separation of lecithin from cephalin, for cephalin though relatively insoluble in alcohol is quite soluble in an alcoholic solution of lecithin. Therefore in precipitating an ether solution of lecithin and cephalin a certain amount of the cephalin remains in solution with the lecithin. Or if the original extract of the tissue is made with alcohol much cephalin will go into solution with the lecithin. MacLean (9) has shown that "lecithin" as customarily prepared can always be separated into true lecithin, which has all of its base as choline and into cephalin with its amino ethyl alcohol base, together with other impurities. He has devised a method fully described in his article by which he has obtained lecithin with all of its base as choline. The method involves the formation of the cadmium compounds of lecithin and cephalin from the alcoholic solution of these phosphatids as obtained from the egg yolk. The separation is made on the basis of the insolubility of the lecithin cadmium chloride compound in ordinary ether, the cephalin cadmium compound being soluble. Pure lecithin is recovered from the cadmium compound by boiling with ammonium carbonate after the manner recommended by Bergell (10). Lecithin prepared by this method when tested in the usual manner exhibited no thromboplastic activity. Attempts made to recover the cephalin from its cadmium compound were unsuccessful.

CONCLUSIONS

1. Cephalin when prepared as pure as possible exhibits marked thromboplastic activity, as indicated by its effect in increasing the thrombic action of fresh serum.
2. Cephalin exhibiting this reaction has been prepared from the liver, heart and brain.
3. The other phosphatids that have been described, lecithin, cuorin, heparphosphatid and sphingomyelin have no thromboplastic action.
4. Evidence is presented to show that this property of cephalin is not due to adherent impurities, but is a characteristic property of the cephalin itself.

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THE RAPIDITY WITH WHICH ALCOHOL AND SOME SUGARS MAY SERVE AS NUTRIMENT

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Alcohol has been commonly considered the most quickly available nutriment. That it is absorbed very rapidly from the alimentary tract into the blood, especially "on an empty stomach," is easily evident to one who has watched the early appearance of psychical symptoms after alcohol is taken. One reason for its early absorption is due to an appreciable proportion being absorbed in the stomach; Nemser (1) in a dog found 20 per cent of the alcohol given was so absorbed.

As the respiratory quotient offers a means of recognizing when alcohol displaces in whole or in part the other nutriments burned in the body, I have endeavored to find out with men how soon after taking alcohol (30 cc.), appreciable amounts of it are being burned. I have further extended the work to include similar experiments with the more common sugars, glucose, levulose, sucrose, lactose and maltose.

The different materials utilized by the body, if burned alone, give the following combustion or respiratory quotients: carbohydrate, 1.00; protein, 0.81; fat, 0.71; while the respiratory quotient of alcohol is 0.667. Commonly, the respiratory quotient of a normal subject, twelve hours after taking food, varies from 0.75 to 0.90 according to the varying proportions of fat and carbohydrates available and utilized in the body. If alcohol be taken, the respiratory quotient is lowered by reason of the substitution of some of the carbohydrate and probably also of the fat by alcohol in the material katabolized. Table 1 shows the average drop in the respiratory quotients in a large number of experiments made by me on men at the Nutrition Laboratory during the first two or three hours after alcohol was taken.

It may be noted in the table that the higher the initial respiratory quotient, the larger the fall after alcohol; thus either a larger amount of alcohol is katabolized to displace the other nutriments, or the alcohol

TABLE 1

Average respiratory quotients following the giving of 30 cc. of alcohol

RESPIRATORY QUOTIENT BEFORE GIVING ALCOHOL	RESPIRATORY QUOTIENT AFTER GIVING ALCOHOL	DROP DUE TO ALCOHOL
0.90	0.805	0.095
0.85	0.795	0.055
0.80	0.775	0.025

shows a preference to displace a greater proportion of carbohydrate than fat, when the initial quotient is high.

In this study, the respiratory quotients were obtained by the method of Tissot (2) the gas analyses being made on the portable Haldane apparatus (3). A mouthpiece with Tissot valves was used. The rubber tubing, joining the expiratory valve with the spirometers, was made as short as possible (air content 200 cc.), passing to a T-stopcock so that the expired air could be instantly changed from one spirometer to another. In order to find as closely as possible the time when a change in the respiratory quotient occurred, the individual periods for the first fifteen minutes following the taking of the alcohol or sugar were of three to five minutes duration.

The routine of the experimenting when alcohol was given was as follows. The subject came to the laboratory at about 8.00 a.m., having had no breakfast. He lay quietly on a couch for at least thirty minutes. Then two or three respiratory experiments were made with the Tissot apparatus, each of about ten minutes duration, to obtain the normal respiratory quotient of the morning. The subject then sat up for a few seconds and drank as a control about 200 cc. of cold cereal coffee. The subject lay down again and respiration experiments began as soon as possible; these covered periods from 1 to 4, from 4 to 7, from 7 to 10, and from 10 to 15 minutes after the coffee was taken. After ten minutes intermission, there was another period of ten minutes. Then a mixture of cereal coffee and 30 cc. absolute alcohol, the total equalling 200 cc., was drunk and similar experiments made after the alcohol as before. The control experiments were to note if taking liquid of itself affected the quotient.

The results of the experiments with alcohol are given in table 2. Eight experiments were made with five subjects. The taking of the liquid, both the alcohol and the control solution, caused an immediate rise in the quotient with all the subjects except E. It was especially marked in subject A where an average quotient of over 1.00 was obtained in the first to fourth minute regardless of whether alcohol or cereal coffee had been taken. So in fixing the exact time when alcohol begins to play a prominent enough part in the metabolism to lower the respiratory quotient, it is necessary to compare the control and alcohol experiments. This rise in the quotient was probably due to a stimulation of the respiratory center of short duration (with a resulting pumping out of carbon dioxide from the body) induced by the taking of liquid.

With subject A, a distinct lowering of the respiratory quotient did not occur until the tenth to the fifteenth minute after alcohol was given; with subjects B and D a similar period was necessary; subject C showed a lowered quotient in the seventh to the tenth minute, while with subject E one finds a marked lowering as early as the fourth to the seventh minute. Thus one may observe that the time between the taking and the beginning of combustion of alcohol varies with different subjects; with some subjects the combustion may begin 4 to 7 minutes after taking alcohol, and varies with others up to 10 to 15 minutes, by which time all the subjects observed gave evidence of burning alcohol.

When sugar is fed, it replaces fat and protein in the katabolism, and in consequence one finds a rise in the respiratory quotient. Sugars thus afford one an opportunity, in the same way as alcohol, of finding how soon after taking they begin to be burned in appreciable amount in the body. In interpreting an increased respiratory quotient, when carbohydrate is given, one is forced however to admit the possibility that the rise is due wholly to the conversion of carbohydrate into fat unaccompanied by an actual combustion of the carbohydrate.

Therefore, experiments, similar in nature to those with alcohol, were made on one subject (E) who alone had shown no change in the respiratory quotient after the control solutions, to obtain data as to the rapidity with which various sugars—glucose, levulose, sucrose, maltose and lactose—began to be burned in sufficient quantities to displace other material and thus affect the respiratory quotient. The plan of experimenting was the same as with alcohol, except that control experiments were not made on the same day as the sugar experiments and that more experimental periods each day were made after sugar was given than after alcohol. One hundred grams of the sugar[1] was made up with water and cereal coffee to about 300 cc. and could be drunk

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[1] An amount of sugar (about 45 grams) isodynamic to the 25 grams of alcohol would doubtless have been preferable.

TABLE 2

Respiratory quotients after a control solution and after 30 cc. alcohol

SUBJECT	BODY WEIGHT	CONTROL							ALCOHOL					
		Date	Prel.	1-4 mins.	4-7 mins.	7-10 mins.	10-15 mins.	25-35 mins.	Prel.	1-4 mins.	4-7 mins.	7-10 mins.	10-15 mins.	30-40 mins.
	kilos													
A.....	54.2	February 27, 1915	0.875	0.990	0.900	0.870	0.880	0.850	0.865	0.975	0.885	0.810	0.825	0.825
		March 1, 1915	0.890	0.965	1.050	0.965	0.900	0.890	0.890	1.030	0.950	0.955	0.895	0.790
		March 12, 1915	0.915	1.065	0.870	0.850	0.895	0.935	0.920	1.055	0.940	0.950	0.780	0.825
		Average	0.895	1.005	0.940	0.895	0.890	0.890	0.890	1.020	0.925	0.905	0.835	0.815
B.....	48.8	April 26, 1915	0.865	0.855	0.840	0.840	0.840	0.845	0.855	0.930	0.810	0.830	0.835	0.795
		May 7, 1915	0.840	0.865	0.850	0.865	0.850	0.855	0.850	0.905	0.855	0.870	0.830	0.805
		Average	0.850	0.860	0.845	0.855	0.845	0.850	0.855	0.920	0.830	0.850	0.830	0.800
C.....	65.2	December 3, 1914	0.860	0.905	0.865	0.840	0.855	0.855	0.860	0.890	0.850	0.810	0.775	0.755
D.....	66.3	April 24, 1915	0.825	0.845	0.795	0.800	0.825	0.825	0.825	0.865	0.800	0.810	0.800	0.800
E.....	67.0	December 5, 1914	0.890	0.875	0.865	0.860	0.840	0.855	0.875	0.860	0.815	0.820	0.840	0.780
		Average of all subjects	0.865	0.900	0.860	0.850	0.850	0.855	0.860	0.910	0.840	0.840	0.820	0.795

quite easily and rapidly by the subject; generally about 25 to 50 cc. of water was taken immediately afterwards to remove the excessively sweet taste from the mouth. The results of the experiments are given in table 3 and the respiratory quotients are plotted in figure 1.

The rise in the respiratory quotient after levulose and sucrose is distinctly rapid, being very marked even as early as the fourth to the eighth minute. There is a rapid rise also with lactose, but not to so marked a degree. With glucose and maltose, the definite rise in quotient does not occur until the twentieth to the thirtieth minute after the sugar was taken, although there is a small rise with maltose a little earlier.

That maltose behaves so similarly to glucose is to be expected from its being a disaccharide made up of two molecules of glucose. Sucrose on hydrolysis breaks down to give one molecule of levulose and one of glucose; probably it is its levulose content which causes the rapid rise in the respiratory quotient. Lactose, giving galactose and glucose on hydrolysis, similarly seems to owe its rapid metabolism to the galactose rather than the glucose moiety of the molecule.

This difference in action of levulose and glucose is quite in agreement with the finding of Tögel, Brezina and Durig (4); they made respiration experiments with these two sugars and found that the respiratory quotient showed a rise earlier after the levulose than the glucose. Another interesting finding in their experiments—namely the uniformly higher quotient after levulose than after dextrose—appears likewise in table 3. These workers concluded that levulose is more easily converted to fat by reason of its respiratory quotient being so often above 1.00. In this respect also, Table 3 shows that cane sugar behaves similarly to levulose and maltose to dextrose. With subject E, lactose yielded a higher respiratory quotient than dextrose, and produced a similar result with another subject at the Nutrition Laboratory; but with three others lactose led to a respiratory quotient no higher than that ordinarily found with dextrose. Lusk (5) has found little or no rise in the respiratory quotient after giving lactose to dogs.

These results with the sugars, although made upon but one subject, are substantiated in practically every detail except as above noted with lactose by a number of unpublished experiments made in another connection by Mr. L. E. Emmes and myself at the Nutrition Laboratory under the direction of Dr. F. G. Benedict, and thus make it clear that there is a fundamental and distinct difference in the metabolism of the various sugars in man and that from a nutritional point of view one

TABLE 3

Experiment with Subject E. Respiratory quotients.

DATE		AVERAGE OF PRELIMINARY	PERIOD FOLLOWING									
Dec. 5, 1914	Control	0.890	1–4 mins. 0.875	4–7 mins. 0.865	7–10 mins. 0.860	10–15 mins. 0.840			21–31 mins. 0.855			
Dec. 5, 1914	Alcohol	0.875	1–4 mins. 0.860	4–7 mins. 0.815	7–10 mins. 0.820	10–15 mins. 0.840			22–32 mins. 0.780			
Jan. 21, 1915	Maltose	0.830	1–3 mins. 0.785	3–6 mins. 0.865	6–9 mins. 0.880	9–13 mins. 0.845	13–18 mins. 0.860	18–23 mins. 0.865	23–28 mins. 0.895	35–44 mins. 0.900		
Dec. 8, 1914	Levulose	0.895	2–5 mins. 0.910	5–8 mins. 0.960	8–11 mins. 1.075	11–15 mins. 1.085	15–20 mins. 1.075	20–25 mins. 1.105	25–30 mins. 1.055	36–44 mins. 1.085		81–89 mins. 0.975
Dec. 29, 1914	Sucrose	0.885	1–4 mins. 0.835	4–7 mins. 0.950	7–10 mins. 1.030	10–14 mins. 1.085	14–19 mins. 1.125	19–24 mins. 1.145	24–29 mins. 1.135	29–34 mins. 1.110	48–56 mins. 0.995	82–90 mins. 1.025
Dec. 11, 1914	Glucose	0.870	1–4 mins. 0.815	4–7 mins. 0.910	7–10 mins. 0.855	10–15 mins. 0.865	15–20 mins. 0.865	20–25 mins. 0.895	25–30 mins. 0.910	40–49 mins. 0.940		79–87 mins. 0.965
Jan. 4, 1915	Lactose	0.845	1–4 mins. 0.915	4–7 mins. 0.875	7–10 mins. 0.880	10–14 mins. 0.900	14–19 mins. 0.940	19–24 mins. 1.015	24–29 mins. 1.015	41–51 mins. 1.010		83–92 mins. 0.950

must recognize the possible necessity of differentiating between them in their *action* and *use.* One might say in brief that levulose and sometimes galactose, judging from the respiratory quotient, shows a tendency or preference to change to fat in the body, while glucose tends to change to glycogen and be stored as such.

A study of figure 1 shows clearly that levulose, sucrose, probably lactose and possibly maltose give indications by the respiratory quotients of being metabolized, and in all probability burned by the fourth to the seventh minute after ingestion "on an empty stomach," which is quite as rapidly as alcohol in the same subject and much quicker than alcohol in four other subjects. Although alcohol is no sooner available as a food, that it will be burned in preference to sugar when the two are taken together has been shown by Tögel, Brezina and Durig (4).

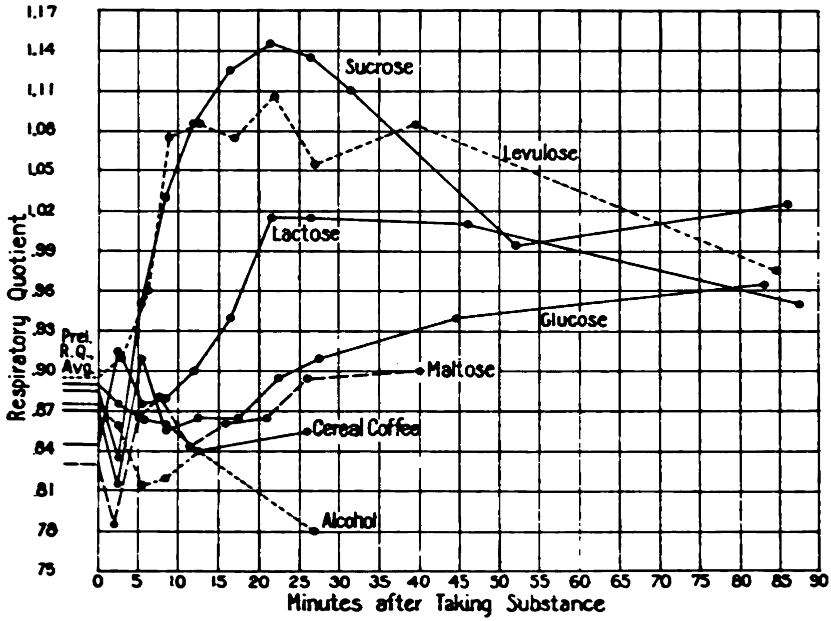

Fig. 1.

SUMMARY

1. By determination of respiratory quotients in periods of 3 and 4 minutes duration upon subjects without breakfast, it was concluded that

a. Alcohol begins to be burned in appreciable quantity in from 5 to 11 minutes after taking; with some subjects the combustion began more quickly than with others;

b. Sucrose, lactose and levulose begin to be burned quite as soon as alcohol, if not sooner;

c. Glucose and maltose are not utilized as food as soon as the other sugars or alcohol, approximately twenty to thirty minutes elapsing before their combustion plays an important part in the metabolism.

2. There is a distinct difference between the metabolism in men of glucose and of levulose and galactose as shown by a study of the gaseous exchange, especially the respiratory quotients.

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UNILATERAL DESTRUCTION OF THE SEMICIRCULAR CANALS IN CATS

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In a previous paper (3), dealing with the physiological anatomy of the falling reflex of cats, a number of observations upon cats subjected to a unilateral removal of the semicircular canals were reported. These former observations were all made as acute experiments, and the rotatory reaction was tested on recovery from ether. In order to determine the permanence of such a lesion, and to ascertain a possible compensation due to assumption of function by other organs, a series of cats was prepared with similar unilateral destruction of the semicircular canals. These observations were continued in some cases over a period of approximately three months; it is the purpose of this paper to present the records of these animals.

Although many workers in physiology have been concerned with the function of the semicircular canals in lower animals (notably pigeons), similar recorded experiments in the form of unilateral destruction of the labyrinth of mammals have been few. Several observations extending over a considerable period of time have been made upon birds and amphibia chiefly. Pigeons, several months after the destruction of one labyrinth, according to Ewald (2), are distinguished with difficulty from normal pigeons. As they are able to perform perfectly their ordinary functions, special tests must be used in order to bring out the slight permanent changes, such as the position of the head, and the preference for standing on the leg of the non-operated side. In regard to frogs with only a single intact canal-system, the same author mentions the fact that the peculiar rotation of the head remains for a long time, decreasing only in its intensity as time goes on. When, however, the frog is disturbed, the original abnormal position is assumed again in its former degree. In some as yet unpublished work by Rosencrantz (4), pigeons with the three canals on one side cut, still showed after a period of six and a half months, some slight residuals of the original lesion; there was not an absolutely complete recovery. Wilson and Pike (6) report that in mammals, e.g., dogs and monkeys, upon the removal of the semicircular canals of one side, certain postural phenomena only, such as the torsion of the head to the injured side and a leaning, particularly of the anterior part of the body to this side, remain permanently.

Since it is not practicable to reach the labyrinth by the same method in every species of animal, many different methods for doing so have been devised. For these experiments the procedure of Wilson and Pike for destroying the canals in cats was adopted, with the modification, however, that a median, dorsal, longitudinal skin incision was made, the superior auricular muscle reflected, and the origin of the temporal muscle laid back from the bone, so as better to preserve the function of these muscles. The cats were operated upon under full ether anaesthesia, strict surgical cleanliness was observed, and the wound was repaired by suturing with silk thread. In the spacious cages in which the cats were kept after the operation, ample opportunity for walking, jumping and climbing was provided.

Before detailing the results noted, it will be well to make clear the terms used in describing the turning reactions, i.e., "turn to the right," and "turn to the left." By saying that a cat turns to the right, for instance, is meant, that if the cat were standing upright on its hind feet it would turn on its long axis in the same direction that a man would turn when turning to his right. Similarly with the term, "turn to the left." Or, in other words, when the cat is standing on its four feet, or turns in the air starting from a position with its feet directed upward, a "right turn" would be one in which, looking at the cat from the cephalic end, the turn of the body would take place in a clockwise direction around the long axis of the body; in a "turn to the left" the turn would be in an anti-clockwise direction. When it is said that an animal "falls on its right side," or "towards the right side," the animal in doing so really turns to the left around its long axis.

Directly after the operation, upon complete recovery from the ether, the reactions of the cats with destruction of the semicircular canals on one side corresponded exactly with those noted in the previous paper. There was a horizontal nystagmus of both eyes to the opposite side, a partial rotation of the head away from the lesion, and an ataxia, especially of the head, variable in degree. The animals reclined always upon the operated side, resisting strongly rotation away from the lesion. Upon being dropped they rotated in the air so as to land upon their feet normally, although requiring a greater distance in which to inaugurate and also to perform the rotatory movement. The turn was always to the right with left-sided lesions, and to the left with right-sided lesions. The majority of animals immediately after recovery from anaesthesia will not rotate in the air while blinded, perhaps because they have not yet learned to trust the impressions received from the intact labyrinth.

The picture presented by one of these cats eighteen to twenty-four hours after the operation was quite constant. The animal showed little desire to walk, but when it did so, it either went in a circle towards the side operated upon; or when it attempted to walk in a straight line, its legs were noticeably abducted, more markedly on the side not operated on than on the affected side. During this progression the whole side of its body was held much lower on the operated side than on the other. This sagging suggested a weakness or loss of tonicity of the muscles on the affected side. Very often, in walking, the animal suddenly fell towards its affected side, i.e., if the right side were the side that had been operated upon, the body would turn to the left around the long axis; and vice versa. It did not, however, tend to continue rolling over and over about its long axis across the floor as Ewald's rabbits did with similar lesions, but it soon got on its feet again and started to walk. However, after taking a few more steps forward it usually fell over on its side again, invariably to the side of the lesion. It was frequently noted that when such a cat, sitting or standing quietly, suddenly shook its head rapidly, the whole body would suddenly fall over towards the affected side. This phenomenon could be brought out also by shaking the head of the animal rapidly from side to side for a few seconds. When the shaking was stopped the animal immediately fell over on its side.

When an animal with a unilateral vestibular lesion was allowed to jump off a chair it would land fairly accurately on its feet, but immediately upon striking the floor the body of the animal would fall over to the side on which the canals had been destroyed. Upon regaining its feet the animal would then start to walk off either to the right or to the left respectively, depending upon whether the right or the left canals had been operatively interfered with.

The attitude of the head of the animal while sitting or standing quietly or while walking was very characteristic and corresponded exactly to the position of the head assumed by frogs as pictured by Ewald, and by mammals in Wilson and Pike's article. The head is rotated around the long axis of the body so that the ear on the affected side is inclined downward, and the nose directed over towards the sound side. This attitude was never changed and was increased to a considerable extent when the cat lapped milk or performed other tasks requiring muscular effort. It was sometimes increased to such an extent in these instances that the side of the head with the lesion was held parallel with the floor.

All the animals still had a horizontal nystagmus to a varying degree after eighteen hours from the time of operation. At times it was rapid, and at times it was slower and coarser. The quick phase of the nystagmus was always towards the non-operated side. Invariably, however, after twenty-four to thirty-six hours, it had completely disappeared.

The responses obtained from a cat with one intact labyrinth to dropping through the air upon a soft bed of straw were very consistent. In every case the animal was first held with its back down and its feet pointing straight upward. If it were an animal, for example, with the right labyrinth removed, rotation was always to the left; the landing on its feet was quite accurate. If this same animal were held so that its feet were in a horizontal plane, with the side of its body and head on which the labyrinth was destroyed (that is, the right in this case) parallel with the floor, it twisted through 270 degrees or more to the left in order to land on its feet. Sometimes it turned too far, going through more than 270 degrees and landing on the side of its body. In every case, however, it made the rotation to the left. Animals with a left-sided lesion reacted in a similar manner, except that they invariably turned to the right. Hence it must be assumed that these experimental cats, on falling, always turned away from the lesion.

When blinded by a hood placed over the head, cats, with one-sided lesions created twenty-four hours or more previously, reacted by turning in the same way as when not blinded. Such a rotation of blinded cats rarely occurs immediately after the operation, but in the short period of twenty-four hours the animal apparently learns to rely upon impulses derived from the one intact labyrinth. With the lesions on the right side in such an animal the turn was always to the left, and vice versa (even if by doing so it was necessary to go through more than 270 degrees in order to secure a perfect landing). But now it was also seen that such animals very often—much more frequently than the non-blinded cats—went too far in their twisting, striking the straw on their sides. Vision, of course, as recorded in the former paper, aided the animal to determine accurately its position in space. One cat when blinded, was seen to make two complete revolutions (720 degrees) through the air before striking the straw. Such extreme rotations require considerable heights for their accomplishment.

The acquirement of the ability of a cat with but one intact vestibule to rotate in the air, on falling, even when blinded is but one indication of a gradual new adaptation of such an organism to its environment. In this instance the recovery of function on the part of one canal-system took place invariably within twenty-four hours. A more gradual recovery was noted in the muscular reactions of all of these cats with one-sided lesions. As was recorded in the previous note, a cat with a unilateral destruction of the semicircular canals required a fall of a considerable distance before the normal rotation was started, if the test were made as an acute experiment shortly after the animal had recovered from the surgical anaesthesia. In these observations, however, the cats twenty-four hours after the operation were noticed to have acquired the power of inaugurating the rotatory response in much more rapid a manner. Thus a fall of 2 to 3 feet was sufficient to allow them to make the necessary twist through 180 degrees. Further acquirement of this ability was a rather slow process, and it was not until two to three weeks after the operation that these animals showed an almost total recovery of their ability to initiate the typical rotation within a very short distance. None of the animals, however, during the three months' duration of observation made a complete recovery. The best explanation of this slow recovery probably concerns not the acquirement of a more acute function on the part of the receptor system, but rather deals with a gradual, but never wholly complete, resumption of normal motor function. Such an explanation would undoubtedly coincide with Ewald's original dictum concerning the relation of the vestibular apparatus to muscular tonus.

With the return, then, of more or less muscular reaction, the rotation of a blinded cat with a one-sided destruction of the vestibule could be foreseen as an example of the power, and possibly of the need, of such an animal to maintain a continuous revolution about the long axis of the body. As recorded above, such a cat was observed to rotate through 720 degrees in the course of a fall of moderate height. Presumably this animal on a much greater fall would have maintained a continuous rotation about its long axis, differing in this regard from the animal with the more acute lesion in its greater muscular power and coordination. This suggests that cats when deprived of their ocular sensations and with but one intact vestibule possess an inherent tendency towards this rotation about the long axis. But another factor undoubtedly influences such continuous revolution of the animal. This factor probably concerns the character of the physical environment to which the animal is immediately subjected. For if such a blinded animal be placed upon the ground, the inevitable reaction consists not in the rotation but solely in a struggle to remove the blinding hood. Deprived, however, of its firm and customary basis for support, as in the air, the single unbalanced canal-system exerts an overpowering and almost inevitable stimulus for the rotation about the long axis of the body. Such a rotation, however, in an unblinded cat seems limited merely to the necessary turning which will protect the animal from injury and allow it to land squarely on its feet. Thus it may be assumed on this hypothesis that a blinded cat with a unilateral destruction of one set of semicircular canals possesses a tendency towards the rotation about its long axis only when in an unstable medium, such as obtains on falling through the air.

This hypothesis was tested by placing the unilateral cats in another unstable medium—water. Five cats, not blinded, two with right lesions, and three with left lesions, were placed in a tub of water, deep enough for them to swim around in without having their feet touch bottom. The results were very striking in that they all started to swim, but after a few strokes the side of the body with the lesion went down in the water and the other side came up. In doing this their heads became submerged, which deprived them of the use of the eyes, and now they began to revolve continuously around the long axis—to the left in those with right-sided lesions, and to the right in those with left-sided lesions. This rapid, continuous revolving of the body lasted as long as the animals were permitted to remain in the water. Interesting was the fact that two of these animals, 59 and 67 days respectively after the operation, were able to swim and climb out of the tub as long as they kept their heads above water; as soon as the heads became submerged they began revolving about the long axis. In this connection Wilson and Pike report in a protocol, that a dog which had had the right labyrinth destroyed a year previously, when taken out to swim, "sometimes turned over and over in the water before starting, but usually came to the surface when thrown in and started for the bank without rotation or turning in a circle."

In the foregoing paragraphs some mention has been made of the gradual change in reaction of these cats with unilateral destruction of the labyrinth. Thus, the failure of the nystagmus to persist for over thirty hours after the creation of the lesion, was recorded. The tendency to walk about in a circle has been observed to persist only for the first few days. Likewise the gradual return of an almost complete muscular ability was pointed out. Certain reactions, however, do seem to endure unchanged at least throughout the period of three months. These reactions concern the inevitable tendency of the animal to rotate away from the lesion about the long axis when placed in an unstable medium. The other characteristic phenomena of such an animal deprived of one labyrinth are chiefly postural in character, and these, in a way similar to the gradual return of the muscular power, come to approximate the normal, but in no sense must this return be considered as a total resumption of the normal functions. Thus, the sagging of one side of the body on walking is gradually overcome but never completely. The animals seemingly tend to lean towards the operated side. In the same category must be included the abnormal position of the head with the depression of the ear towards the operated side, and the incomplete rotation of the nose. This persists probably to a greater extent, and for a longer period than does the loss of muscular power. In every case a very considerable vestigial remnant of this postural reaction was evident three months after the initial lesion. Interesting in this connection is the fact that on exertion or during any special effort, this abnormal deflection of the head would reappear and persist to a marked degree, but for merely a few moments. It was noted that shortly after the initial operation these animals when shaking their heads fell towards the affected side. Gradually, in the course of several weeks, this spontaneous phenomenon grew less and less frequent and the tendency to fall less and less pronounced. So it would seem that the animals subjected to a unilateral destruction of the semicircular canals tend slowly to recover from the initial effects of such a lesion; the recovery in no sense can be taken to be complete.

SUMMARY

In conclusion it may be stated that cats subjected to a unilateral destruction of the semicircular canals show the following typical responses:

1. During the first twenty-four hours there results a marked horizontal nystagmus to the opposite side; a varying degree of ataxia of the

whole body (especially of the head); and a typical rotation of the head with depression of the ear on the operated side.

2. With the loss of the nystagmus after about thirty hours, and a loss of the tendency to walk in a circle after a few days, there is a very gradual but incomplete recovery of the postural and muscular reactions of the animal.

3. On being dropped in the air, the tendency of these animals is to rotate away from the lesion, and to land accurately on their feet.

4. Blinded cats with one-sided vestibular lesions show after the first twenty-four hours an inevitable tendency towards rotation away from the lesion about the long axis of their bodies. Such rotations have been observed to continue through 720 degrees. Similarly, unblinded cats when placed in water, and with their heads submerged, continuously rotate around the long axis of their bodies.

5. All the rotations, whether falling to the injured side while walking, or rotating while dropping through the air, or rotating while submerged in water, all take place in the same direction around the long axis of the body—to the right in cats with the lesion on the left side, and to the left in those with right-sided lesions.

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A PHOTOGRAPHIC METHOD FOR MEASURING THE SURFACE AREA OF THE HUMAN BODY

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In a study made at the Nutrition Laboratory in 1912 of the metabolism of a man during a 31-day fast, the heat production was calculated in accordance with the usage of physiologists on the basis of per kilogram of body weight and also on the basis of per square meter of body surface (1). As the only method available at that time for computing the body surface was the Meeh formula, i.e., $12.312 \sqrt[3]{\text{body weight}^2}$, it was used in this instance. In the discussion of the results, however, it was stated that owing to probable defects in the applicability of the Meeh formula the absolute values were doubtless erroneous and that even the differences could not be looked upon as entirely accurate. With the development of the Du Bois linear formula (2) for measuring the body surface and the consequent demonstration of errors in the Meeh formula, an effort was made to obtain more probable values for the body surface of the fasting man for subsequent use in recomputing the heat production per square meter of body surface. Unfortunately the measurements made of the subject during the experimental period i.e., the regular anthropometric measurements of the gymnasium, are not those required by the Du Bois formula. Use was therefore made of a series of anatomical photographs of the subject taken at intervals during the fast and a photographic method was developed for measuring the body surface which was based entirely upon the Du Bois linear formula. We were thus able indirectly to take advantage of the greater accuracy of the Du Bois method. The results of this recomputation of the body surface of the fasting man are published in the accompanying article (3), together with a discussion of the heat production per square meter of body surface as computed upon the new basis.

This photographic method for measuring the body surface of individuals was the outcome of an attempt previously made to secure the body surface of infants. At that time certain photographic poses were studied with a view to using them as a possible basis of measurement of the body area of adults (4), but with the Du Bois linear formula it was practicable to simplify these poses considerably. In the development of the photographic method photographs were taken of a considerable number of normal individuals of widely varying weight and body configuration whose body area had been measured by means of the Du Bois formula. The areas shown in these photographs were then accurately measured by the planimeter. From the results obtained with the planimeter and the values for the body surface of the individuals as secured by actual measurement and computation with the Du Bois formula, a formula was developed which could be used for calculating the body surface of any individual from photographs taken of certain poses. A detailed description of the development of the method and the values obtained for the individuals photographed are given in this paper.

GENERAL PRINCIPLE OF THE PHOTOGRAPHIC MEASUREMENTS

As a basic measurement a cross section of the nude body through the extended arms and fingers, trunk and legs was considered to be the closest approximation to the body surface. If the body were as flat as a leaf this area, multiplied by 2, would give the total body surface but the impractical nature of even such a cross section as this is instantly seen when it is remembered that while the cross section of the extended hands and fingers is included in the surface area, the cross section of the feet and toes is not included since they are at right angles to the body. This cross section would furthermore assume equality in surface area on the dorsal and ventral sides of the body. With approximately normal men this would not be too gross an assumption but the mammae in women and the pendulous abdomen of the obese would not be included; hence a profile view was taken with the subjects standing at "attention." Various other profile views were taken with one arm extended and with and without one leg forward of the other to include greater lateral area. A more detailed description of the principal poses, with illustrations, will be given later.

It must be noted at the outset that these photographs are made in a wholly empirical way for obviously the human body is not as flat as a leaf and likewise the profile view does not represent the thickness of the arm but shows only a cross section through the center of the body. Nevertheless, since for comparison the actual body surface could be computed by the Du Bois linear formula from definite body measurements, it seemed feasible to attempt some correlation between the body areas of a number of individuals as measured by the planimeter on photographs showing these poses.

TECHNIQUE

Inasmuch as the outline of the body alone was desired, no attempt was made to differentiate sharply the details of the photographs, particularly as a quick exposure was highly advantageous. The technique for taking the first photographs conformed to the technique employed with the anatomical photographs of the fasting subject. In these a black background was used in the belief that the predominantly light-colored body would give a sharp contrast. Reasonably satisfactory results were obtained in the series of photographs taken in this way although certain dark shadows, particularly on the insides of the legs and portions of the side of the body made it difficult to trace the outline with the planimeter.

The best results were obtained by using as a background a white, semi-transparent screen (a bed sheet) which was placed in front of a window. The camera was located approximately 3 meters from the body and pointed directly toward the window. This procedure gave a sharp black outline against a white background, the resulting print being a fairly good silhouette. But even with this method a few of the pictures showed certain high lights on the shoulders which led us to believe that it would be better to restrict the white field to correspond closely to the form of the body. Theoretically the more nearly the outline on the photograph represents a true reduction of the silhouette the better. To give a suitable scale for measurement, a meter stick was always placed near the body in the same plane as the silhouette and included in the photograph.

Practically all of our pictures were taken with a lens already in the possession of the laboratory, i.e., a Cooke lens, Series V, f/8, 6½ x 8½ inches, with an equivalent focus of 11.2 inches. This was used with a 6½ x 8½ inch plate, the body nearly covering the plate. A series of smaller photographs, taken with a relatively inexpensive Kodak on 4 x 5 inch plates, was also made of one of the subjects. These latter photographs, although requiring more care in planimetering, gave concordant results with the photographs obtained on the large plates. To study the effect of possible distortion, an extraordinarily wide angle lens—a Bausch and Lomb, Zeiss Protar, f/18, Series V, 6½ x 8½ inch, with an equivalent focus of 5$\frac{3}{16}$ inches was used with a 6½ x 8½ inch plate. Even when these distorted pictures were planimetered, values were obtained which did not exceed 9 per cent of those found with the Cooke lens, the values ranging from 3 to 9 per cent. Such an extraordinarily wide angle lens would rarely be used, however, as the photographer would instantly see the distortion. Consequently we may say that with practically any lens giving figures not evidently distorted a high degree of accuracy and concordance can be obtained. The prints were made on carbon Velox paper and when dry were temporarily mounted on a drawing board with thumb tacks; the area was then planimetered.

POSES

The photographs were taken of the subject in three principal poses, which are here designated as poses A, B, and C. Later developments showed that of these pose C is the most useful and accordingly, to illustrate the exact method of taking the pictures, a typical photograph of this pose is given of subject 7 (fig. 1). Furthermore, silhouettes of the same subject in all three poses are given (fig. 2), together with silhouettes of poses A and C for a normal woman subject, no. 17, a very fat man, no. 1, and a very small man, no. 14 (fig. 3). Silhouettes are also given of pose C for all of our other subjects (fig. 4). The scale is indicated in each case by a line showing the length of the meter stick photographed at the same time in the same plane as the part of the body focused upon, i.e., in poses B and C exactly opposite the backbone. To destroy the identity of the subjects, the contour of the face has been obliterated in all of the silhouettes.

Pose A. On the supposition that pose A, representing presumably the largest regular cross-section of the body, would be more particularly the basic measurement to which could be applied a factor of possibly [illegible], special emphasis was laid upon it and a large number of photographs were taken showing the hands and arms in various positions. At first the hands and arms were arranged with the open hands at approximately the level of the ear, the arms spread as nearly as possible in the plane of the medial line of the body. Subsequently it was found that an easier pose and one that gave exactly the same area when measured by the planimeter was that illustrated, i.e., a normal

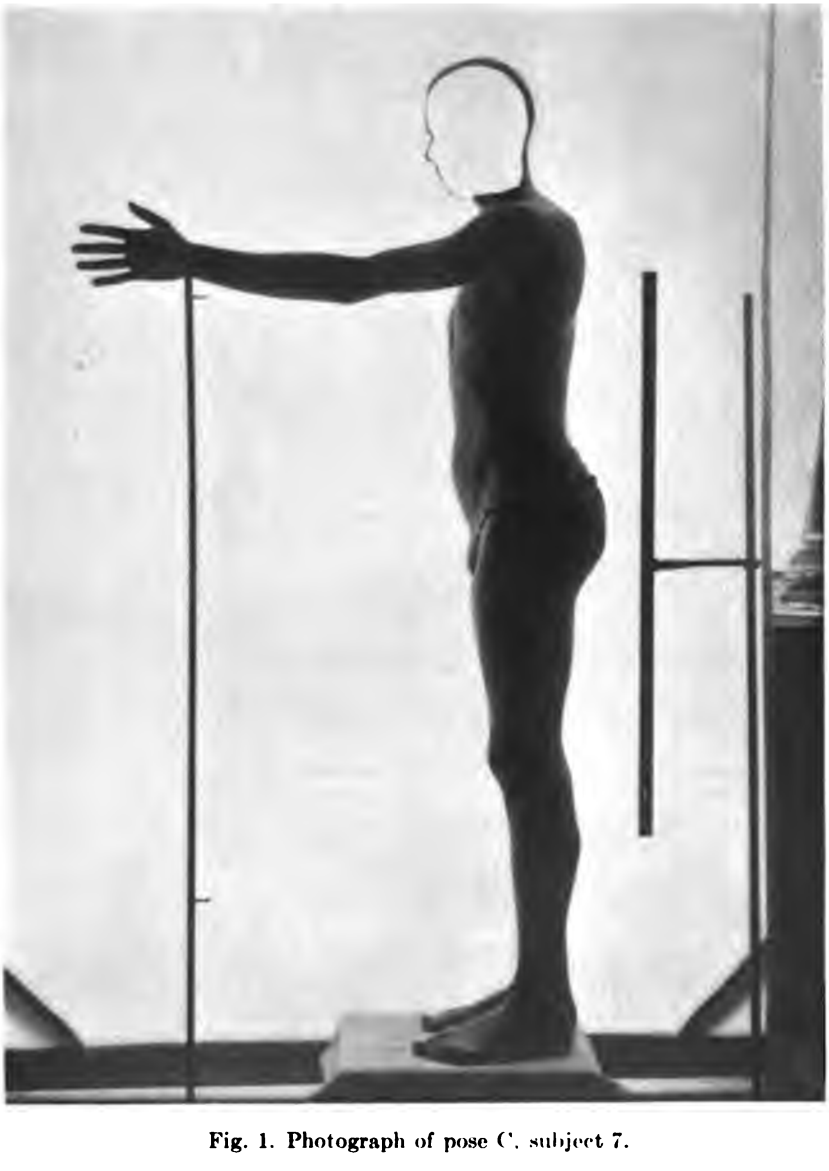

Fig. 1. Photograph of pose C, subject 7.

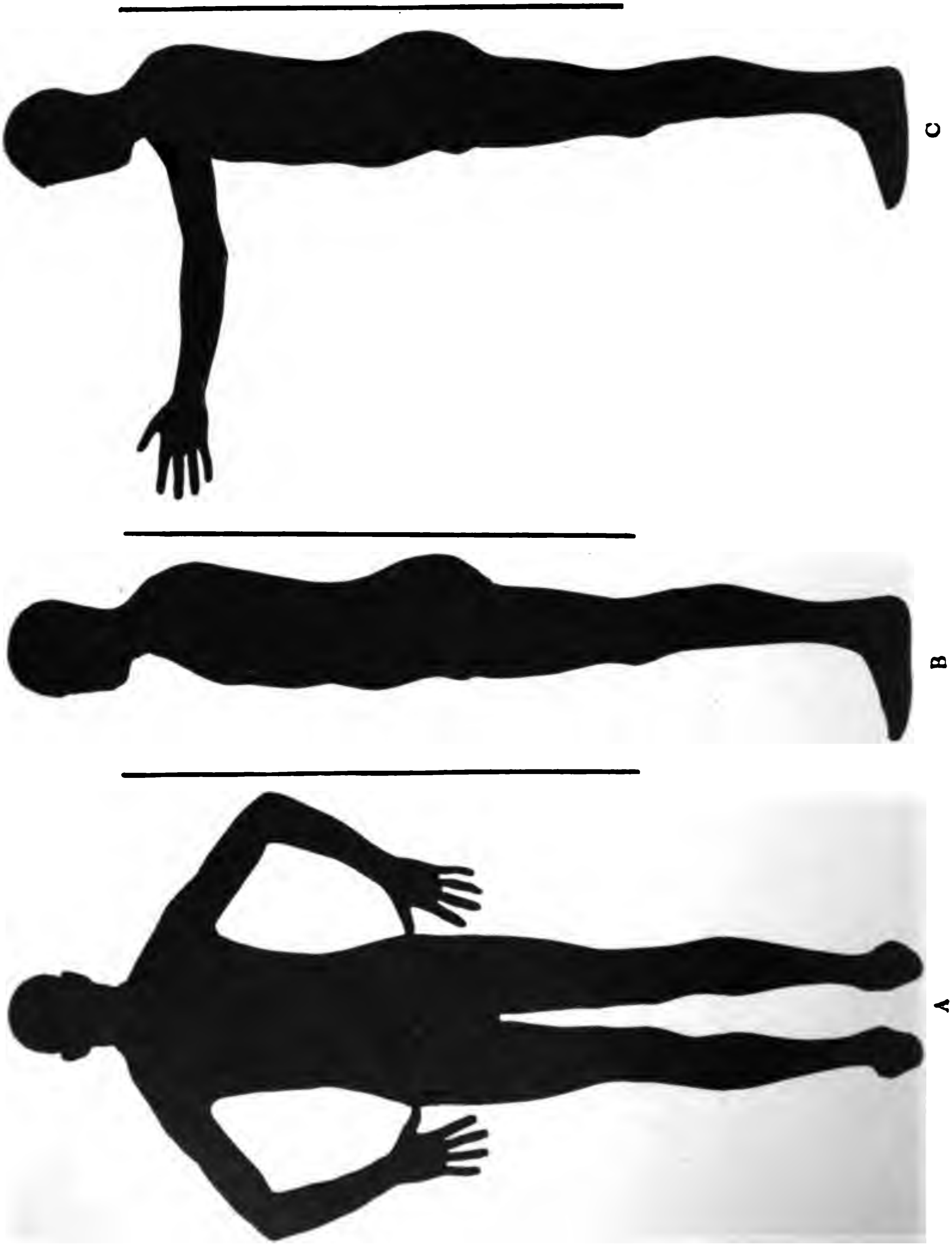

Fig. 2. Silhouettes of poses A, B, and C, subject 7.

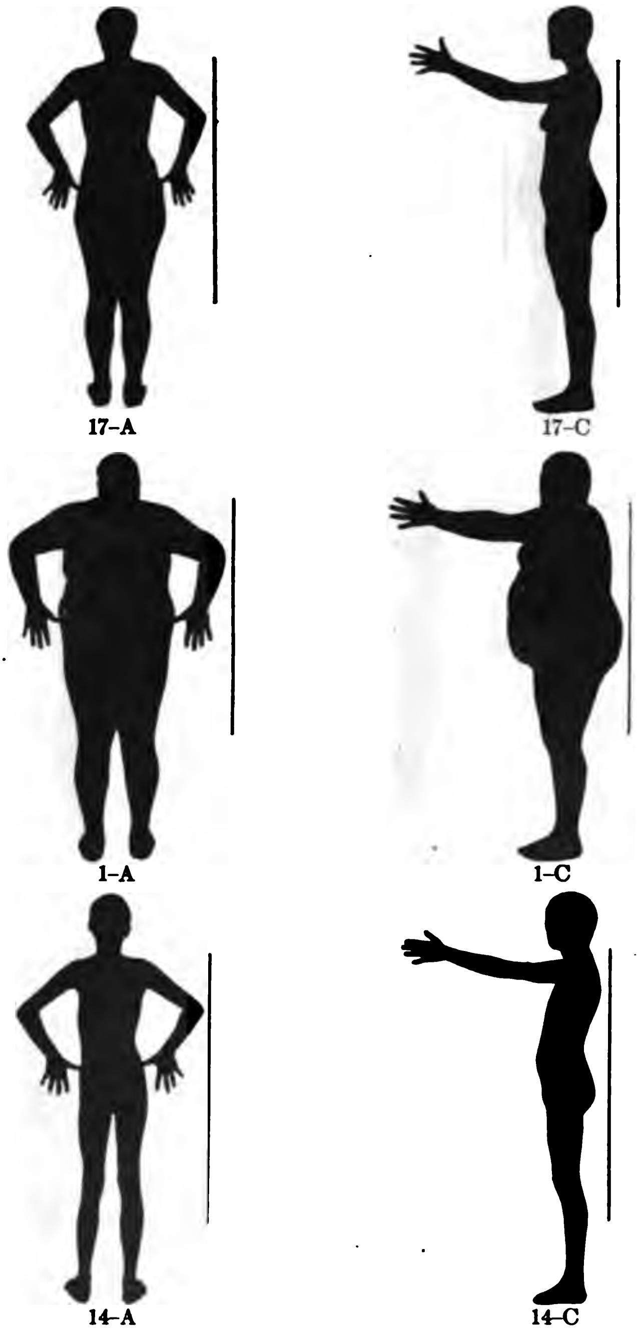
17–A
17–C
1–A
1–C
14–A
14–C

Fig. 3. Silhouettes of poses A and C, subjects 17, 1 and 14.

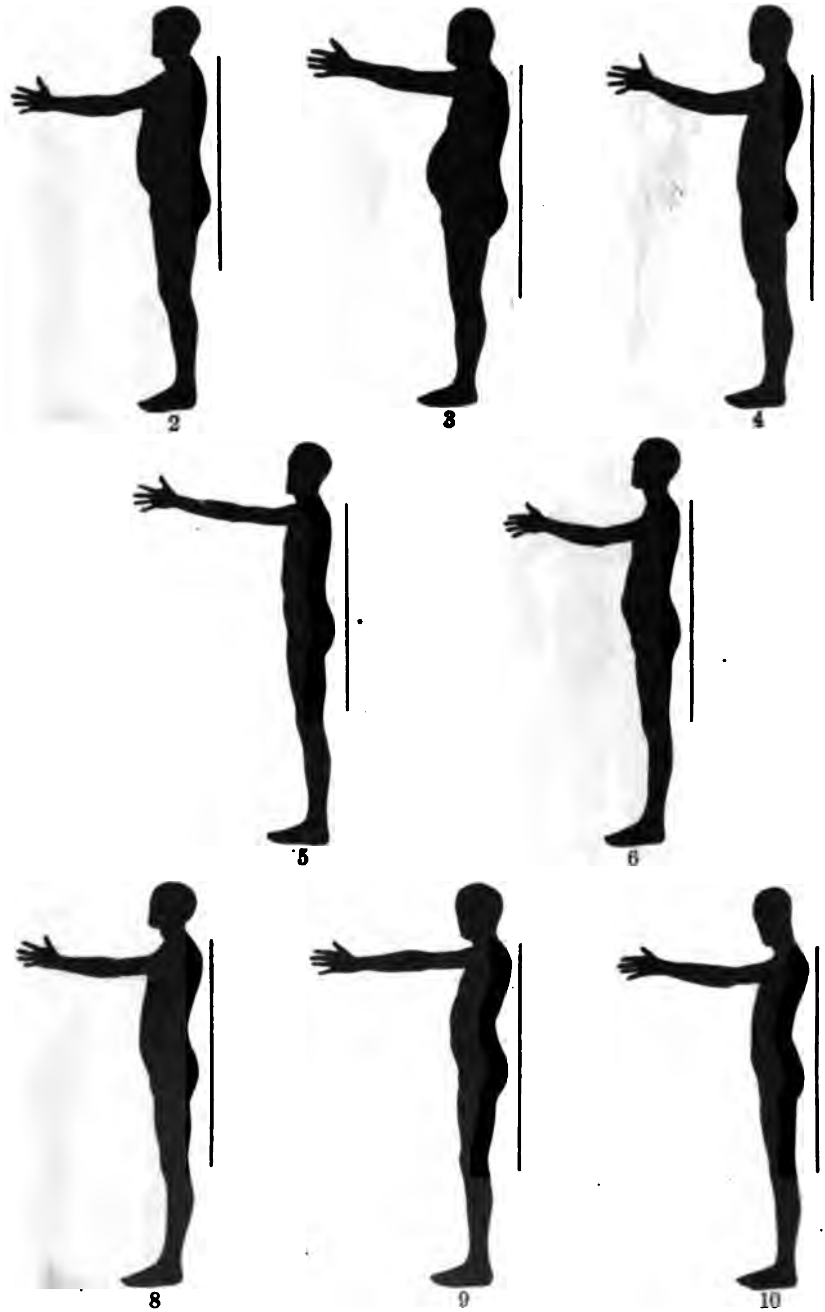

Fig. 4. Silhouettes of pose C for 16 subjects of varying height and weight.

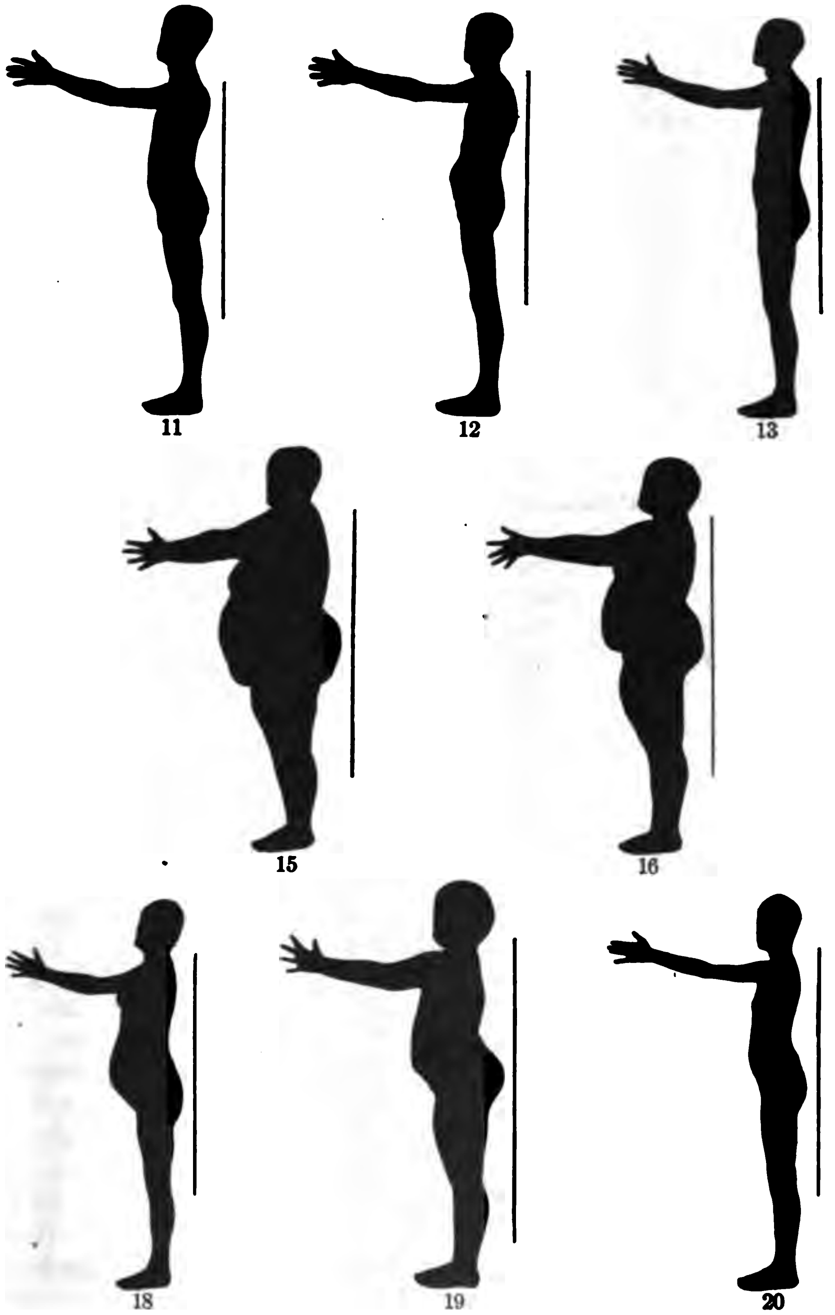

Fig. 4—Continued.

view of the subject with arms akimbo and thumbs resting more or less lightly upon the hips. In planimetering the figure it is important to make allowance for the hair. After very little experience this allowance is readily made and the duplicate measurements with the planimeter show extraordinary accuracy.

Pose B. A profile view of the subject standing at "attention" was selected for pose B. Theoretically this pose shows but one leg. As a matter of fact unless the camera is placed at a very great distance, the other leg will inevitably show, particularly at the feet. Consequently the slight amount of high light on the edges of the leg nearest the camera is distinctly advantageous for indicating the proper outline of the nearer leg for planimetering. In running the planimeter over the photographs of the subject in these poses only the outline of the leg and foot nearest the camera is taken. In adjusting the pose the operator attempts to secure such a position that the leg farthest from the camera will be hidden as much as possible by the leg nearest the camera. With this pose, as with pose A, it is necessary to make allowance for the hair and, with a bearded subject, for the beard. However, even with women, especially when the hair is knotted at the back of the head, no difficulty is experienced in determining the probable contour of the head.

Pose C. Pose B is, however, theoretically unsound inasmuch as it represents the cross section of but one extremity, namely, one leg, while the arms are completely hidden in the shadow of the body. Consequently the subjects were photographed in a third pose, pose C, which differed from pose B in that the left arm was extended in a natural way and the fingers of the hand separated. The original photograph of subject 7 (fig. 1) and the silhouettes of the other subjects in this pose (figs. 2 to 4) show very clearly the position to be adopted. The axis of the arm was directly perpendicular to the plane passing through the limbs and the vertical upright of the body; in other words, the hand should not be brought over into a line opposite the breast bone. With female subjects the arm should be held sufficiently high to show the breast below. In planimetering, the same precautions should be used as with the other poses, i.e., the hair should not be included, and the outline of the leg nearest the camera alone should be used. It was believed that this pose would have special value in indicating some possible relationship between the photographic cross section and the area, particularly in subsequent attempts to secure the surface area of animals in which a cross-section photograph would show the profile of two extremities. Even with pose C it will be seen that the position is purely empirical since it by no means represents a complete cross-section of the body.

In all three poses the question of the separation of the fingers is of minor technical importance. As a matter of fact, tests have shown that regardless of whether the fingers are close together or are separated, the same results are obtained in planimetering. The separation of the fingers, however, illustrates a little more clearly the possibility of considering the body as a series of short cylinders from which the area of the body may be calculated.

RELATIONSHIPS BETWEEN THE BODY SURFACE AS COMPUTED BY THE DU BOIS FORMULA AND FROM THE PLANIMETERED AREA OF SPECIAL PHOTOGRAPHIC POSES

The observations were made with 20 subjects, of whom six were females. The body weight of the men varied from 144.6 to 32.9 kgm. and of the female subjects from 99.1 to 37.1 kgm. All persons measured were 21 years or over, with the exception of two girls (nos. 16 and 19) who were but 10½ and 6½ years old. The data for the age, nude body weight, and height of the subjects are given in columns *a*, *b*, and *c* of table 1. From these data it will be seen that the subjects varied widely in general configuration of body. Thus, the heaviest individual, with a weight of 144.6 kgm. had a height of but 166.2 cm., while the smallest man was also the shortest. Of the women the heaviest, weighing 99.1 kgm., was likewise the shortest. Thus we have with both the heaviest man and the heaviest woman a typical picture of obesity. Of the normal individuals many were rather thin and several were very tall. A number of the men were members of the laboratory staff and had been most carefully measured on numerous occasions. One of the women (no. 17) was an artist's model, selected for normality of figure. Two of the men (nos. 11 and 12) and two of the women (nos. 18 and 20) were diabetics and were somewhat emaciated.

I wish here to express my indebtedness to Dr. E. A. Locke for his kindness in putting me in communication with the obese subject 1, to Dr. F. B. Talbot and his assistant, Dr. J. M. Lee, for their help in securing the coöperation of subjects 16 and 19, and to Dr. E. P. Joslin for the diabetic patients. I am especially indebted to Dr. J. H. Means not only for securing the assistance of the obese subject 15 but for many helpful suggestions. Without the coöperation of the subjects (5) little could have been accomplished and to all I desire to express my thanks, especially to my intimate associates who volunteered and also assisted in the numerous measurements.

Cross section photographs were made of all of the subjects in the poses A, B, and C, and by means of the planimeter and the measurement of the photographed meter stick, the total area shown in the photograph, or the true area of the body in that particular section, was computed. The method of computation was as follows:

Owing to the size of the paper used, it was generally necessary to divide the body into two approximately equal parts by a line passing through the hips. The upper and lower sections were planimetered separately and the sum of these two areas, which on the instrument we used was expressed in square inches, was multiplied by the factor 6.45 for conversion to square centimeters. The actual length of the reduced meter stick was measured in millimeters (generally from 104 to 114 mm.) and the true area obtained by a simple proportion.

Thus, using subject 7 for an illustration, the photograph of pose C, reproduced in figure 1, gave for the lower part of the body 3.11 sq. in., and for the upper part 3.40 sq. in., with a total of 6.51 sq. in. This, multiplied by the factor 6.45, gave a total of 42.0 sq. cm., or, as our result is to be expressed in square meters, 0.00420 sq. m. The meter scale as reduced in the photograph measured 108.25 mm. or 0.10825 m. The proportion used, therefore, was as follows:

$$l^2 : 1^2 :: a : x,$$

l representing the length of the reduced meter scale (0.10825 m.), a the area of the photograph expressed in square meters (0.00420 sq. m.), and x the surface area of the section of the body shown in pose C (0.358 sq. m.). The general equation would therefore be:

$$x = \frac{a}{l^2}$$

The calculated areas based upon the various photographic measurements for poses A, B and C, expressed in square meters for each of the subjects, are given in columns *d*, *e*, and *f* of table 1. As would be expected, the values for pose A are the largest and the values for pose C are larger than those for pose B owing to the additional area of the extended arm and hand.

Since the entire investigation is based upon an assumption of the fundamental accuracy of the Du Bois formula, each of these subjects was most carefully measured, usually by two, frequently by three individuals, and after numerous personal conferences with Dr. E. F. Du Bois. I would like at this point to express my warmest appreciation of the assistance and great number of suggestions that Dr. Du Bois has given me, both by personally instructing members of the Nutrition Laboratory staff in the technique of measuring and by assisting in clarifying several obscure points in obtaining accurate measurements by this formula. The area of each individual[1] as computed from the Du Bois measurements is given in column *i* of the table. These areas represent the base line to which all the values subsequently computed are referred. The values computed by the Meeh formula are given for comparison in column *j*.

RELATIONSHIPS OF AREA OF PHOTOGRAPHIC POSES TO TRUE BODY SURFACE

When the photographs of the subjects in the three poses A, B, and C were examined certain astonishing relationships were almost immediately noted. Neither pose A nor pose B take into consideration the area of the ends of the body, i.e., the bottom of the feet and the top of the head and shoulders. Furthermore, it is obvious that since the body is not as flat as a leaf, the surface of the body will be larger than twice the area of the cross section shown in A, but how much more remains to be determined. If we consider the body as made up of a series of short cylinders without ends, we might express the relationship between the photographic surface A and the true surface by the formula πA. On the other hand if we take twice the area in pose A and add to it twice the area of the profile view (pose B) we should assume that the body was a rectangle in shape; we should still have no measure of the area of the ends of the body. As a result of these preliminary considerations the following formulas were selected, first, πA, and second, $2A + 2B$. It will be seen that πA represents the surface of a cylinder of the area of the cross section A, without taking the ends into consideration, while $2A + 2B$ indicates a rectangular formation, also without including the ends.

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[1] It is especially important with obese subjects in taking the measurements L and W of the Du Bois formula to make the measurements between perpendiculars. Thus, the distance in the length between the suprasternal notch to the upper border of the pubes with a fat person, if measured by a tape laid directly on the skin, will represent a distinctly curved distance, and hence the length will be too long. Similarly the length W, which is from the upper border of the pubes to the lower border of the patella, should likewise be measured between perpendiculars. Owing to difficulties in locating the superior border of the great trochanter with fat people the value W has been used instead of the value O. Dr. Du Bois has kindly supplied a constant where W is used, this constant being 0.552. See Du Bois and Du Bois; Arch. Intern. Med., 1916, xvii, 863.

The values obtained by using the first formula (πA) are given in column *g* of table 1. A comparison of these values with those obtained with the Du Bois formula as given in column *i* shows somewhat lower values for those computed from the formula πA. On the other hand it will be noted that these values are very much nearer the Du Bois values than are those calculated by the Meeh formula and given in column *j*, these latter values being invariably somewhat higher. The values as computed by the second formula ($2A + 2B$) and given in column *h* of the table show usually a striking uniformity with those obtained with the first formula, πA, and likewise a closer approximation to the Du Bois values than the Meeh values. This uniformity in the values as computed by the formulas πA and $2A + 2B$ appears throughout practically the entire series, the greatest discrepancies being shown with the two obese individuals, nos. 1 and 15. Numerous other interesting relationships were established between the various cross-section photographs, their perimeters, the Du Bois formula, and the area computed from the Meeh formula.

As an inspection of the values obtained by means of the formula πA shows that the results were invariably somewhat lower than those found with the Du Bois linear formula, it is obvious that a factor somewhat larger than π is essential for computing the true surface area from the cross-section photographs of the subjects in pose A. To obtain this factor the values found by the Du Bois formula were divided by the values given in column *d* for the areas of the photographs of the pose A. The results of this computation are given in column *k* and are in all cases higher than π, ranging from 3.21 to 3.89, with an average value for the 20 subjects of 3.48. Using this constant alone, therefore, we may compute the body surfaces of this heterogeneous group of individuals, with very wide variations in body contour, weight, and height, and obtain results varying only from + 11.6 to −7.7 per cent from the true body surface, a majority of the values lying within ± 4 per cent.

Using the area of the photograph in pose B as the basis, and dividing the total areas as computed by the Du Bois formula by the areas of the pose B photographs as found with the planimeter, we obtained the

TABLE 1

Measurements of the body surface of men and women by various formulas

SUBJECT NO.	AGE	NUDE BODY WEIGHT	HEIGHT	AREA SHOWN IN VARIOUS POSES			TOTAL BODY SURFACE				DU BOIS AREA DIVIDED BY			TOTAL BODY SURFACE 5.02 C	DIFFERENCE BETWEEN DU BOIS AND PHOTOGRAPHIC METHODS
				A Back	B Side	C Side, with arm extended	Photographic measurements		Du Bois formula	Meeh formula $12.312\sqrt[3]{W^2}$	A $i+d$	B $i+e$	C $i+f$		
	(a)	(b)	(c)	(d)	(e)	(f)	πA (g)	2A+2B (h)	(i)	(j)	(k)	(l)	(m)	(n)	(o)
	years	kilos.	cms.	sq. m.	sq. m.	sq. m.	sq. m.	sq. m.	sq. m.	sq. m.				sq. m.	per cent
Males															
1	30	144.6	166.2	0.692	0.467	0.536	2.17	2.32	2.69	3.39	3.89	5.76	5.02	2.69	0
2	45	87.1	182.2	0.618	0.360	0.422	1.94	1.96	2.10	2.42	3.40	5.83	4.98	2.12	+1
3	39	84.8	166.3	0.566	0.335	0.399	1.78	1.80	2.04	2.38	3.60	6.09	5.11	2.00	−2
4	23	70.4	170.8	0.570	0.308	0.381	1.79	1.76	1.91	2.10	3.35	6.20	5.01	1.91	0
5	21	69.0	185.4	0.573	0.321	0.385	1.80	1.79	1.95	2.07	3.40	6.07	5.06	1.93	−1
6	48	60.4	179.7	0.522	0.285	0.343	1.64	1.61	1.74	1.90	3.33	6.11	5.07	1.72	−1
7	23	59.8	172.9	0.518	0.298	0.358	1.63	1.63	1.74	1.88	3.36	5.84	4.86	1.80	+3
8	36	59.1	176.1	0.509	0.295	0.338	1.60	1.61	1.71	1.87	3.36	5.80	5.06	1.70	−1
9	27	57.5	173.5	0.490	0.282	0.341	1.54	1.54	1.73	1.83	3.53	6.13	5.07	1.71	−1
10	26	57.1	174.3	0.521	0.283	0.332	1.64	1.61	1.67	1.83	3.21	5.90	5.03	1.67	0
11	24	53.6	167.5	0.487	0.267	0.329	1.53	1.51	1.59	1.75	3.26	5.96	4.83	1.65	+4
12	55	49.5	166.4	0.464	0.263	0.320	1.46	1.45	1.63	1.66	3.51	6.20	5.09	1.61	−1
13	37	48.0	164.8	0.434	0.260	0.307	1.36	1.39	1.47	1.63	3.39	5.65	4.79	1.54	+5
14	24	32.9	148.1	0.349	0.202	0.239	1.10	1.10	1.19	1.26	3.41	5.89	4.98	1.20	+1
Females															
15	50	99.1	144.7	0.540	0.363	0.421	1.70	1.81	2.09	2.64	3.87	5.76	4.96	2.11	+1
16	10½	86.5	144.3	0.533	0.332	0.383	1.67	1.73	2.00	2.41	3.75	6.02	5.22	1.92	−4
17	24	56.6	159.6	0.481	0.264	0.321	1.51	1.49	1.65	1.81	3.43	6.25	5.14	1.61	−2
18	56	49.1	156.6	0.413	0.253	0.299	1.30	1.33	1.53	1.65	3.70	6.05	5.12	1.50	−2
19	6½	44.3	126.8	0.361	0.228	0.259	1.13	1.18	1.29	1.54	3.57	5.66	4.98	1.30	+1
20	35	37.1	151.7	0.395	0.220	0.263	1.24	1.23	1.31	1.37	3.32	5.95	4.98	1.32	+1
Average......	......	......	......	......	......	....	......	......	....	....	3.48	5.97	5.02		

constants recorded in column *l*. As would naturally be expected in view of the smaller area shown in pose B, we find that the constants are somewhat higher than those for pose A, ranging from 5.65 for subject 13 to 6.25 for subject 17 (the artist's model), with an average value of 5.97. The variation from the average is even less for pose B than for pose A, being at the most approximately ± 5 per cent. With either series of constants, but especially with the second, values much nearer to the true body surface may thus be obtained than with the Meeh formula.

When we compute the values from the areas obtained from the planimetered surface of pose C, we find even more concordant results (see column *m* of table 1). These values range from 4.79 with subject 13 to 5.22 with subject 16, the obese girl; the average value is 5.02. The extraordinary constancy exhibited by these values is certainly most difficult to explain. It may be shown more clearly if we use this constant of 5.02 to compute the surface area from the planimetered area of pose C for each subject and compare the results (5.02 C.) with the values obtained from the Du Bois measurements and the Du Bois formula. This comparison is made in columns *n* and *o* of table 1 in which we find that of the 20 subjects three show values in practically perfect agreement with the true area as determined by the Du Bois formula, ten vary only 1 per cent, three 2 per cent, one 3 per cent, two 4 per cent, and one 5 per cent. We see, therefore, that the maximum error in using this constant is 5 per cent; the average error with this factor is ± 1.6 per cent. In view of the fact that the data for the subjects given in table 1 are arranged in order of the decreasing body weight and hence approximately in order of the decreasing body surface, it is astonishing to note the distribution of the agreement of the computed values with the true body surface. The largest man and the smallest man both show nearly perfect agreement; the same happens to hold true for the largest and smallest of the relatively few female subjects. It will be seen from these computed values that a singularly constant relationship exists between the true body surface as measured by the Du Bois formula and the area of a silhouette photograph of a side view of the body with the left arm extended. In addition to supplying an accurate basis for body surface measurements the silhouette gives ocular evidence of the general configuration of the body, a factor of especial value in observations on obese, emaciated, and abnormal individuals.

SUMMARY

Primarily with the object of securing measurements for a subject who had undergone a 31-day fast, a photographic method was developed to establish the relationship between the area of the body computed from certain definite photographic poses (particularly a side view with arm extended) and that actually measured by the Du Bois linear formula. A method for securing accurate measurements of the body area resulted. Comparisons between the photographed areas and the body surface as computed from the Du Bois linear formula show, even with the most diverse configurations of body, a constancy rarely observed in anatomical measurements or in computed ratios based upon such measurements.

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(5) Since a number of these individuals have had their metabolism studied and reported elsewhere, it is of interest to note that nos. 2, 3, 6, 8, and 13 correspond to F. G. B., F. A. R., Dr. S., L. E. E., and T. M. C., respectively, in the paper by **Benedict**, **Emmes**, **Roth**, and **Smith**. Journ. Biol. Chem., 1914, xviii, 139, and No. 15 to Mrs. McK. in the paper by **Means**. Journ. Med. Research, 1915, xxxii, 121.

THE RELATIONSHIP BETWEEN BODY SURFACE AND HEAT PRODUCTION ESPECIALLY DURING PROLONGED FASTING

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One of the earliest outcomes of an attempt to explain the relatively higher food demands of small animals of a given species as compared with those of larger animals of the same species was the formulation by Bergmann (1) of the generalization that the heat production of a body is proportional to its surface. No direct experimental evidence bearing upon this particular point appeared until 40 years later, when Rubner (2) in his classical research on animal calorimetry, maintained that the heat lost from living organisms is essentially constant per unit of body surface. While this constancy in relationship between body surface and heat production is the dominant note in Rubner's writings, it is well to remember that in several instances he writes as if a causal relationship between body surface and heat production was by no means thoroughly established. On the other hand, Richet (3) in his writings published about the same time, stated without qualification, that the heat production of a warm-blooded organism is determined by its body surface.

The apparent establishing by Rubner of a simple mathematical relationship between body surface and total metabolism became naturally the subject of much discussion. The range in the animal kingdom over which this supposed law extended was indeed astonishing, the interesting computations of E. Voit (4) appearing to show that animals ranging in size from a 2-kilogram fowl to a 441-kilogram horse have essentially the same heat production per square meter of body surface, namely, 970 calories per 24 hours.

It is true that marked variations from the general law were soon noted but attempts were made to explain these discrepancies by the nature of the integument, the density of the fur and hair coverings, and variations in the amount of body surface exposed; in this connection we frequently find in the text of the earlier writers such statements as the following from Rubner: "Wärmeabgebende Fläche und Hautfläche sind zwei sehr verschiedene Dinge." (5)

The members of the Zuntz school in Berlin were extremely cautious about accepting the general thesis as to the relation between body surface and metabolism. As possibly representative of this we may quote the following expression from Magnus-Levy and Ernst Falk (6).

> Welche inneren Ursachen diesem Gesetze zu Grunde liegen, ob die behauptete Abhängigkeit eine directe oder indirecte ist, haben wir hier nicht zu erörtern; wir wollen bloss prüfen, ob unsere Zahlen mit dieser Annahme übereinstimmen oder nicht. Eine ausführliche kritische und theoretische Erörterung der obigen Lehre findet sich in v. Hoesslin's (7) gedankenreichem Aufsatze: "Ueber die Ursache der scheinbaren Abhängigkeit des Umsatzes von der Grösse der Körperoberfläche."

Von Hoesslin's paper attracted considerable attention but the general view that the body surface determines the metabolism has predominated essentially since that time. The relationship between the body surface and Newton's law of cooling was perhaps more emphasized by the members of the French school than by the Germans; yet when the influence of environmental temperature was studied, difficulties were immediately encountered and we find certain interesting considerations brought out by d'Arsonval (8) in an article giving a summary of his numerous investigations covering a period of six years. In discussing the fact that certain animals show abnormal relationships between the environmental temperature and their body temperature, he introduces the following significant sentence:

> Cela tient évidemment à ce que la surface rayonnante *physiologique* de l'animal n'est pas constante comme sa surface *physique*. Aux basses températures, le phénomène se complique d'une constriction vasculaire périphérique, qui restreint considérablement le pouvoir rayonnant de l'animal à égalité de surface *physique*. Cela montre que la connaissance de la surface géométrique d'un animal est insuffisante pourqu'on en puisse déduire la perte par rayonnement; il faut encore tenir compte de l'état de la circulation périphérique.

Finally, a hint of the extremes to which too strict an adherence to the law of body surface may lead us is given in Pütter's contention (9) that we should take into consideration the "active" surface, i.e., the cell surfaces of the various organs of the body, Pütter maintaining that the energy consumption is proportional not to the body surface but to the area of the lung surface.

In this connection it should certainly be pointed out that a not inconsiderable proportion of the total heat output of the day is given off from the lungs through the warming of the air and through the vaporization of water. No system of body surface measurement with which we are thus far familiar takes into consideration this rather important factor. It has been computed as a result of a large number of experiments with human subjects at rest, either with or without food,that from 11 to 12 per cent of the total heat output of the day is required to heat the inspired air and to vaporize the water in the lungs(10). It has likewise been computed that only about 75 per cent of the total heat is given off by radiation and conduction, and approximately 12 per cent by water vaporized from the skin. Certainly the 11 to 12 per cent of the total heat produced which is required to warm the inspired air and to vaporize the water in the lungs—two factors that have no relation whatsoever to body surface—is not, so far as we are aware, considered in any discussion of body surface in its relation to basal metabolism.

It is thus clear that there has been at no time a fixed interpretation of the relationship between body surface and heat production for even its most ardent advocates have at times called attention to noticeable abnormalities. Unfortunately many modern writers are not so conservative in their expressions as to the cause for this relationship between body surface and heat production as were the earlier writers. In his deservedly oft-cited contribution on respiration to Schaefer's Physiology Pembrey says (11):

> Now small mammals and birds have a temperature equal to or even higher than that of large animals of the same classes; and, on account of the relatively greater surface which they expose for the loss of heat, they must have a relatively far greater production of heat than the large animals, for there is generally no marked difference in the protective coat of fur or feathers.

Although Lefèvre specifically states that the application of the law of Newton to living animals is illusory (12) nevertheless in his discussion of the production of heat per unit of surface the following statement appears:

> La production chez l'homéotherme est *en équation* avec la perte calorique. Or, à pouvoir émissif égal, la déperdition est évidemment proportionnelle à la surface rayonnante. La production calorique (c'est-à-dire, chez l'organisme en équilibre et au repos, le besoin d'énergie) est donc proportionnelle à l'étendue de la surface totale du corps (13).

Furthermore, Professor H. P. Armsby, undoubtedly the foremost writer in America on animal nutrition, states:

> The results which we have been considering show that in general the emission constant, i.e., the rate of heat emission per unit of surface, is substantially the same in small and large animals, and that the greater loss of heat in the former case is met by an increased production. In this aspect the effect is simply an extension of the influence of falling temperature, the increased demand for heat being met by an increased supply, so that the extent of surface appears as the determining factor of the amount of metabolism (14).

Even in current (1915) literature we find such statements as the following:

> Rubner's law, to quote from Lusk, is that "the metabolism is proportional to the superficial area of an animal. In other words, the metabolism varies as the amount of heat loss at the surface, and its variance in accordance with this law is necessary for the maintenance of a constant temperature" (15).

In a popular textbook on nutrition we also find:

> Since the body loses heat in proportion to the extension of its surface, it is not strange that this is the determining factor for the metabolism (16).

Between 1885 and 1915 practically no direct comparison of measured body surface and metabolism was made. The technique for body surface measurements is very complicated and investigators as a whole have accepted the measurements of Meeh (17) in which the body surface is equal to the two-thirds power of the weight multiplied by a constant which, for man, is 12.312. The constants for animals, which figure so prominently in E. Voit's (18) remarkable calculations apparently demonstrating the universality of the law, have rested undisturbed in the textbooks for many years, together with that for human organisms, and have rarely been questioned. On the other hand they have frequently had to bear the brunt of the explanation for seeming discrepancies in the application of the law, a good illustration of this being the attempted explanation of the difference in metabolism between atrophic and normal infants on the ground that, with atrophics, there is a disproportion between weight and surface (19).

The recent observations of Moulton (20) are also not without significance in connection with the possibility of errors in the factors for obtaining the true body surface.

Recognizing the probable errors in the values given by the Meeh formula and stimulated by a desire to correlate basal metabolism and body surface more exactly, D. and E. F. Du Bois (21) have developed an exact linear formula which is destined to revolutionize completely our conception of body surface. With this formula it may be said that, given certain body measurements, the body surface of any human individual may be calculated with an unusual degree of accuracy; furthermore, a photographic method for measuring the body surface which has recently been developed in the Nutrition Laboratory (22) fully confirms the fundamentality of the formula. In the hands of the Du Boises and those trained under them, the measurements are certainly made with accuracy and expedition. Unfortunately they do not give one any conception of the general configuration of the body measured, even when accompanied by records of weight and height, the two factors most commonly reported in metabolism studies. That the Du Boises have succeeded in substituting for the admittedly uncertain Meeh formula a method of measurement having a degree of accuracy rare in anatomical measurements is a cause for congratulation. Their contribution to body surface measurement awakens new interest in the relationship between body surface and metabolism.

It is the consensus of opinion of physiologists that the preferable basis of measurement for the metabolism of human organisms is not per kilogram of body weight but per square meter of body surface. The evidence secured in this laboratory leads us to believe that this is due not to the loss of heat from the body surface but to the fact that the body surface is a better index of the general law of growth, in full conformity with the principle laid down by v. Hoesslin (23).

The specific purpose of determining the body surface, therefore, is that it may be used for computing the heat production per unit of surface as a basis for comparison between various individuals. In this method of comparison a value for basal metabolism which is derived from the average results obtained with a large number of persons has been suggested as the "normal" or "standard" value to be applied to each individual case. It will be seen that a prerequisite for such a method is an exact knowledge of the surface area. This method also assumes a universality in the application of the law of surface area.

The belief in this universal applicability of the so-called law of surface area is so widespread that we find a strong tendency among physiologists to apply the law upon all occasions. If there is a law of surface area, i.e., if the heat production of the living animal is proportional to its surface area, we should examine the results of metabolism studies closely to find out to what extent this law may be applied and more especially to find out if the metabolism of human individuals maintains a strict proportionality to body surface.

Several researches carried out in this laboratory will, it is believed, contribute extensively to our knowledge of the relationship between body surface and metabolism. In accumulating experimental evidence on the normal metabolism of men and women at the Nutrition Laboratory, we have strongly contended against the generally accepted view of a causal relationship between body surface and heat production, and an attempt was made in a series of papers recently published to clarify the situation by an analysis of the basal metabolism of 89 men and 68 women (24). The primary object of this series of papers was to discuss the probable factors controlling basal metabolism and it was frankly stated that the loss of heat from the body surface could not be considered as the determining factor. Unfortunately these papers have been interpreted, in part at least, as discouraging any efforts to secure accurate measurements of body surface. This was far from the intention of the writers. The accurate measurement of body surface is an important physiological problem, solved in a highly creditable manner by E. F. and D. Du Bois. The use of body surface as an index of basal metabolism is a wholly different problem. That there is a relationship between the body surface and metabolism no one can deny. That this relationship is causal or sufficiently exact to be designated as a physiological law may be fairly questioned.

As a result of the interpretation of the values obtained with normal men and women, certain factors, such as sex, age, and athletic training, were demonstrated as affecting the basal metabolism, even on the basis of per square meter of body surface, thus showing that there are distinct exceptions to the so-called body surface law. It is of interest to note that the existence of three of these factors has been admitted by other writers.

In an experiment carried out upon a dog which was confined to the laboratory for several months and which did not lose weight, it was proved (25) that the metabolism decreased very considerably (19 per cent). When the dog was again allowed country life, her metabolism returned to essentially its original value but the body weight was unchanged. Here evidently there was a constancy in the body surface but a noticeable difference in the heat production per square meter of body surface. We therefore have still another demonstrated factor affecting basal metabolism and vitiating the universal applicability of the body surface law.

As an index of the caution exhibited by Rubner in presenting this law we have the following interesting statement:

> Das Oberflächengesetz gilt unter allen physiologischen Lebensbedingungen, zu seinem Beweise ist aber sinngemässe Voraussetzung, dass nur Organismen mit gleichartigen physiologischen Leistungen, was Ernährung, klimatische Einflüsse, Temperament und Arbeitsleistung trifft, verglichen werden (26).

As a matter of fact this particular quotation has been cited in adverse criticism of conclusions from this laboratory regarding the comparison of the basal metabolism of normal and atrophic infants. In other words, judged upon this basis, the body surface law could not be expected to hold with individuals under different physiological conditions.

At the time that our analysis of normal data was made, our only knowledge of body surface was that supplied by the antiquated formula of Meeh; in view of the development of later and more exact methods of measurement, a re-analysis of the large mass of metabolism measurements is certainly necessary. If correction is made for the gross errors in the Meeh formula it is conceivable that the discrepancy between heat production and body surface may be reduced or, indeed, wholly disappear. But the revision of the earlier formulas throws discredit upon the previous selection of constants and if the errors in the Meeh formula are so large as those pointed out by the Du Boises, one may also reasonably question the formulas for lower animals; it is thus probable that the final computations of E. Voit may likewise need a radical revision.

It should be noted, however, that in our analysis of normal material and in the comparisons of diabetics and normal subjects, vegetarians and non-vegetarians, athletes and non-athletes, another method of comparison was used which is entirely independent of surface area measurements. This may be designated as the "group system" of comparison, by which the metabolism of groups of persons of like height and weight are compared on the fundamental assumption that individuals of like height and weight have the same general growth relations. Thus far no method of measurement that has been proposed affects the reliability of such a procedure and no existing experimental evidence diminishes in any way one's confidence in this form of comparison.

With the new-born infants that we have studied in this laboratory, many of the complex factors, such as age, variations in muscular activity, changes in diet, etc., which apply to adults, also the variations in growth, are eliminated; hence it seemed desirable to study the influence of variations in the body surface upon the metabolism of these infants. Using the Lissauer formula, in which the constant differs somewhat from that in the Meeh formula, we found no obvious relationship between normal and atrophic infants and no approximation to a physiological "law" was observed until infants between one day and six days old were studied. At that age it was found that if the heat production per square meter of body surface per 24 hours, as computed by the Lissauer formula, was divided by the length of the infant a relatively constant factor of 12.65 calories was obtained.

We still believe that the method of group comparison used for diabetics, athletes, vegetarians, and men and women is fundamentally sound. For a true knowledge of the heat production in various diseases there is no reason why the group system of comparison should not be employed as but little definite information may be expected until a considerable number of cases of the particular disease to be studied is obtained. For a study of the metabolism during prolonged fasting, however, it is impracticable to obtain a sufficient number of cases to permit the use of this method; consequently a 31-day fasting experiment made in this laboratory has a particular interest in this connection (27). The differences in the metabolism shown in this fasting experiment during complete muscular repose were so striking and the bearing of these variations upon the law of surface area is so great that an accurate determination of the body surface of the man as the fast progressed is essential for comparative purposes since it is obviously impossible to make a group comparison for each stage of the fast.

While from time to time constancy or equality in the condition of nutrition has been emphasized as a prerequisite when the law of surface area is to be applied, nevertheless this has by no means been generally considered as a necessity, and we find current practice tends to accept the universality of this law irrespective of whether the individual is poorly or well nourished. Information with regard to the metabolism of individuals that are well or poorly nourished is, for the most part, obtained by observations on different individuals. But during prolonged fasting we may observe in the same individual changes in the plane of nutrition fully comparable to those roughly characterized as poorly or well nourished. It is thus seen that during prolonged fasting simultaneous measurements of the body surface and the basal metabolism of the subject have an unusual value.

A study of the relationships of body weight, body surface, and basal metabolism during fasting is all the more important when it is remembered that it is commonly believed that the fasting animal rapidly adjusts itself to the minimum metabolism. The results of earlier experiments on the dog, the cock, and the guinea pig (28) indicate that per kilogram of body weight the fasting metabolism is constant. With the fasting man studied in this laboratory the metabolism per kilogram of body weight did not indicate constant values, although the measurements were made under identical conditions of muscular repose. This lack of constancy on the basis of per kilogram of body weight led to the computation of the metabolism on the basis of per square meter of body surface, the body surface being determined by means of the Meeh formula. But these values likewise showed no constancy.

During so long a fast as this there was naturally a marked change in the body weight and a change in the configuration of the body. According to tradition any disturbances in the relationship between body surface and heat production could be attributed to a possible disturbance in the relationship between body weight and the computed body surface. Although in the discussion of the results due consideration was given to the possibility of such a disturbance in the relationship between body weight and body surface as the fast progressed, yet, using Meeh's formula for computing the body surface—the only one available at that time—we found a large loss in the heat production per square meter of body surface during the progress of the fast, with variations towards the end which indicate a distinct change in the stimulus to the metabolic activity of the whole body. While it is highly improbable that an error in the Meeh formula would make it so inapplicable to the same individual at the beginning and end of a 31-day fast as to invalidate completely the differences in the metabolism found on different days, nevertheless, as was pointed out in the discussion of the subject, (29) the metabolism of this man per square meter of body surface as measured by the Meeh formula is undoubtedly erroneous so far as its absolute value is concerned and, in all probability, is somewhat erroneous so far as the differences are concerned.

In view of the variations in the metabolism per square meter of body surface shown by this fasting subject when computed on the Meeh basis, it seems all the more important to obtain accurate measurements of the body surface of this man by the new method of the Du Boises. The anthropometric measurements made of the subject at the time of the fast (30) do not give the data required by the Du Bois linear formula. It has been possible, however, by employing a photographic method recently developed in the Nutrition Laboratory (31), to recompute the body surface from a series of anatomical photographs taken of the fasting man at intervals during the study (32). The body area shown in the photographs was measured with an accurate planimeter. Similar measurements were made of photographs of a number of normal individuals whose body area had been determined by the Du Bois formula. With the results obtained from these photographs and from the planimetered photographs of the fasting subject, the body surface of the fasting man could be obtained by a simple proportion.[1] We were thus able by an indirect method to revise the values for the body surface at different stages of the fast.

Since the metabolism of the subject of this 31-day fasting experiment has a special significance in its bearing upon the legality of comparing directly the basal metabolism of a general group of normal, robust individuals with that of a group of individuals emaciated by disease, the heat production per square meter of body surface of our subject during his 31-day fast has been recomputed on the basis of these new values for body surface and the results obtained are here discussed.

[1] It should be stated here that the technique and poses used in taking the photographs of the fasting man were not those finally decided upon as the most satisfactory for the photographic method. Accordingly the photographs of normal subjects used for comparison were those taken in the earlier stages of the development of the photographic method in which the poses were similar to those used with the fasting man. This fact is specially emphasized, since photographing the body against a white sheet, as described under "Technique" in the description of the method published in the accompanying article (Benedict, this Journal, 1916, xli, 275), was a later development of the photographic method of securing a measure of the body surface.

As this manuscript goes to press the latest contribution of the Du Boises to the technique for the estimation of body surface has appeared in the form of a most ingenious height-weight chart (Amer. Journ. Med. Sci., 1916, cli, 781), based fundamentally upon their linear formula but in the final computations considering simply height and weight. The height-weight chart thus gives tacit assent to the validity of our earlier assumption that individuals of like weight and like height have the same surface area and are hence perfectly comparable. A comparison of the measured surface obtained on our subjects with the values found in the height-weight chart shows that for individuals of average height and weight and, indeed, for thin individuals the agreement is very satisfactory. Unfortunately, for the obese this is not invariably the case. It should be stated, however, that the Du Boises have not projected their curve to include distinctly obese individuals. It is of especial interest in this connection to note that the areas on the Du Bois height-weight chart for the subject of the prolonged fast, A. L., agree perfectly with those found by the photographic method.

HEAT PRODUCTION DURING PROLONGED FASTING

In this study of a fasting man absolutely no food was taken by the subject for 31 days and only about 900 cc. of distilled water was given him daily. The heat production during the night was measured directly with the bed calorimeter for each of the 31 nights. It was likewise computed indirectly from the carbon-dioxide excretion and oxygen consumption during the same period. It was immediately observed that as the fast progressed there was a very noticeable difference in the heat production from night to night. This would naturally be expected, since the individual grew smaller each day, the initial weight being not far from 60 kgm., while the final weight was about 47.5 kgm. On the general assumption that a small animal will give off less heat than a large animal, a quantitative decrease in metabolism would thus be expected. The results of the heat measurements of this individual have been considered in detail elsewhere and reference must be made to the original publication (33) for the methods of calculation and for the discussion of the heat production per kilogram of body weight in which an attempt was made to reduce the observations of each night to a common standard. Only the metabolism per square meter of body surface is considered in this paper.

The subject of our 31-day fasting experiment may be considered to have been at the beginning of the fast a normal individual, i.e., a person in presumably good health; he would therefore be perfectly comparable to those individuals without obvious pathological lesion who have also been studied in this laboratory. During the progress of the fast he remained (so far as ocular evidence is concerned) for the most part physiologically normal. The strength tests made with the hand dynamometer during the fast showed practically no change with the right hand and but a slight fall wth the left hand, although there was an almost immediate evidence of fatigue in the first two or three days of the fast. While there was naturally a certain amount of weakness observable in the last few days, an idea of the strength of this man may be obtained from the fact that after having been without food for 31 days he spoke extemporaneously before a body of physicians for approximately three-quarters of an hour, standing during the whole period and vigorously gesticulating. Later in the day he actually sang and danced at times in anticipation of the closing of his long fast. It is thus clear that we have here to do not with a fasting man who is in the last stage of emaciation and in a moribund condition but with an individual who, judged from ocular evidence, would appear not at all unlike the normally emaciated type of individual.

Furthermore, the body temperature during the fast did not materially alter. Indeed, his average body temperature in the bed calorimeter experiment on the night of the last day of the fast was but 0.3°C. below that of the night of the second day, a difference in body temperature that is wholly comparable with that observed by Lusk with his dog during the pre-cage confinement, just after prolonged confinement, and after the dog's return from the country. In other words, we have here no marked disturbance of the body temperature.

Although this man's pulse was distinctly lower at the end of the fast than at the beginning, it will be seen that he underwent the 31-day fast without a great loss of muscular strength or a material alteration of the body temperature. In spite of the absence of these disturbing factors the metabolism when computed on the basis of per kilogram of body weight showed a decided loss as the fast progressed. There was also a loss in metabolism per square meter of body surface; this is shown by the data in table 1, in which are given the nude body weight, the body surface as computed by the Meeh formula and from the measurements of the anatomical photographs, and the heat production per square meter of body surface per 24 hours as based upon the observations with the bed calorimeter during the night. In computing the heat production per square meter of body surface per 24 hours, use has been made of the body surface as computed from both the Meeh formula and the photographic measurements.

It will be seen from the figures given in table 1 that, using as a standard the body surface values obtained with the photographic method, the body surface as computed from the Meeh formula is invariably too large and consequently the heat production per square meter computed from this measure of the body surface is too small. Disregarding the last food day prior to the fast, we find that the heat production per square meter per 24 hours as given in the last column of the table ranges from 927 calories on the third day of the fast to as low as 664 calories on the twenty-first day of the fast, representing a decrease in the heat production per square meter of body surface of 28 per cent. Thereafter there was a distinct tendency for the heat production to increase.

In the absence of any marked change in body temperature the difficulty of considering the loss of heat from the surface of the body as the determining factor in the metabolism of this fasting man is very

TABLE 1

Heat produced by fasting subject during experiments in bed calorimeter at night
(Fasting period 31 days from April 14 to May 15, 1912)

DATE	DAY OF FAST	BODY WEIGHT WITHOUT CLOTHING	BODY SURFACE		HEAT PRODUCED PER SQ. METER PER 24 HOURS	
			By Meeh formula $12.312\sqrt[3]{W^2}$	Computed from photographic measurements	Meeh formula	Photographic method
1912		*kilos*	*sq. meters*	*sq. meters*	*cals*	*cals*
April 13–14		60.87	1.91	1.71*	858	958
14–15	1st	59.86	1.88	1.70	817	904
15–16	2nd	58.91	1.86	1.68	830	918
16–17	3rd	58.01	1.84	1.66*	836	927
17–18	4th	57.22	1.83	1.66	827	912
18–19	5th	56.53	1.81	1.66	764	833
19–20	6th	56.01	1.80	1.65	774	845
20–21	7th	55.60	1.79	1.65	760	825
21–22	8th	55.18	1.78	1.65	790	852
22–23	9th	54.74	1.77	1.65	720	772
23–24	10th	54.25	1.77	1.65*	725	778
24–25	11th	53.94	1.76	1.64	715	767
25–26	12th	53.64	1.75	1.64	712	760
26–27	13th	53.48	1.75	1.63	709	761
27–28	14th	53.22	1.74	1.62	698	749
28–29	15th	52.92	1.74	1.62	649	698
29–30	16th	52.40	1.73	1.61	639	687
April 30–May 1	17th	51.91	1.71	1.60*	642	686
May 1– 2	18th	51.57	1.71	1.60	653	698
2– 3	19th	51.21	1.70	1.60	676	719
3– 4	20th	50.97	1.69	1.60	666	704
4– 5	21st	50.60	1.69	1.59	625	664
5– 6	22nd	50.22	1.68	1.59	653	690
6– 7	23rd	50.00	1.67	1.59	655	688
7– 8	24th	49.70	1.67	1.59*	651	684
8– 9	25th	49.40	1.66	1.58	637	670
9–10	26th	49.10	1.65	1.57	695	731
10–11	27th	48.78	1.64	1.57	673	703
11–12	28th	48.52	1.64	1.56	676	711
12–13	29th	48.19	1.63	1.55	691	726
13–14	30th	47.79	1.62	1.54	698	734
14–15	31st	47.47	1.61	1.53*	701	737

* Body surface for days on which photographs were obtained, i.e., April 13, 16, 23, 30, and May 7 and 14. Other values obtained by direct interpolation.

great. Had the body temperature fallen materially the explanation of the decrease in the heat production could easily be made on the basis of difference in temperature potential. No such explanation is, however, at hand.

The prerequisite conditions for the application of the surface law as outlined by Rubner (34) are somewhat difficult to interpret. If a strict insistence is laid upon like physiological conditions, such as nourishment, climatic influences, temperament, and capacity for work, Rubner would doubtless maintain that the law could not be expected to apply under the conditions of prolonged fasting. Yet these conditions are by no means incomparable with the conditions commonly existing with the majority of pathological subjects with whom enormous variations in the previously mentioned physiological factors are invariably found. A closer examination of the conditions existing during prolonged fasting is not without interest in connection with the treatment by other writers of the results of our studies of the metabolism during diabetes.

In reporting an extended series of observations made in the Nutrition Laboratory on patients with severe diabetes, we compared the metabolism of the diabetics with that found in a series of observations with normal individuals of like height and weight, i.e., of a somewhat thin and emaciated type. As a result of this comparison and the marked difference in metabolism found with diabetics when acidosis was present as compared with that when it was diminished or absent, we asserted that diabetes increased the metabolism approximately 15 to 20 per cent above that of the normal individuals with whom the diabetics were compared. In a discussion of our assertion in which the wholly arbitrary basis of comparison was used of a normal standard value obtained with a large number of individuals of whom the greater proportion were in full vigor, it has been contended (35) that the emaciated diabetics with acidosis showed little or no increase in metabolism. If it is erroneous to apply the surface area law to a normal subject throughout a prolonged fast, it is difficult to see the validity of applying it when there are such marked variations in conditions of nourishment and bodily vigor as existed between the large group of normal persons and the group of emaciated diabetics. In fact, it has been demonstrated that when the diabetics are without acidosis as, for example, when following the remarkable Allen treatment, the metabolism is distinctly lower (36) than with acidosis, so that unquestionably the acidosis *per se* materially increases the metabolism.

With the fasting individual it is evident that, judged by the Rubner specifications, the body surface law may not be expected to obtain; indeed, we find it does not obtain. Nevertheless, it should be pointed out that the differences in the fasting man at the beginning and end of the fast are by no means so great as the differences between pathological individuals, including diabetics, and the average normal vigorous individuals from whom the standard of comparison proposed by other writers has been derived.

With the Meeh formula the results obtained for body surface were clearly questionable, especially when the formula was applied to a body of changing weight; but in view of the accuracy of the Du Bois linear formula, which has been confirmed by the photographic method, we may now accept with full confidence the later measurements of body surface for this individual; hence the values obtained for the heat production as given in the last column of table 1 probably represent with a high degree of accuracy the heat production per square meter of body surface per 24 hours under approximately comparable conditions.

To account for this great difference of 28 per cent in the heat production per unit of surface area, i.e., a physiological measurement that not only is presumed in general to give constant results with all classes of individuals, if not indeed with all warm-blooded animals, but is now specially advocated as the standard for measuring variations in the metabolism in pathological cases, is by no means a simple task. It is certainly clear that the heat production of this individual was in no sense determined by his skin area and the loss of heat from the skin. Variations in body temperature also fail to account for the change in the heat production. The fact that there was in the last week of the fast a rise in the metabolism accompanied by a rise in the pulse rate lends strong credence to the belief that there was present a stimulus to the metabolism which was in a way indicated by the pulse rate; hence we must reiterate (37) our belief that of the numerous factors affecting metabolism, the internal stimulus to metabolic activity is one of the greatest.

SUMMARY

1. An historical consideration of the development of the idea of proportionality between the body surface area and heat production shows that fundamentally it was originally based upon Newton's law of cooling. The idea of a causal relationship between body surface

and heat production is strongly emphasized in foreign writings and distinctly to be inferred from those of a number of American writers. The long existing doubts as to the validity of the older methods for the measurement of body surface have been fully substantiated by the development of the linear formula of the Du Boises, which gives measurements with a high degree of accuracy.

2. Employing a photographic method recently devised for measuring the body surface, the body area of the subject of a 31-day fasting experiment was re-determined and used in recomputing the heat production per square meter of body surface. The values obtained showed a decrease similar to that previously found. The decrease in the heat production per square meter of body surface amounted to 28 per cent, a decrease that can be interpreted only as proof of the inapplicability of the surface area law to subjects with widely varying states of nutrition. This shows it to be impossible to compare a standard value obtained with a large number of robust, normal individuals with that obtained with emaciated diabetics, and thus supplies strong proof of the legitimacy and practicability of the group system of comparing pathological cases with normal individuals of like height and weight, i.e., of general anatomical and morphological similarity.

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THE INTER-AURICULAR TIME INTERVAL

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INTRODUCTORY

In 1910, Wybauw (1) and Lewis (2) working independently, demonstrated that in the mammalian heart, the point of initial excitation corresponds to the location of the sino-auricular node. This finding has been confirmed by Eyster and Meek (3) and others. Although Erlanger (4) and his co-workers have placed a different interpretation on the records of the above named investigators, the view is generally held that the normal seat of impulse formation, is the sino-auricular node and more particularly the head of this node. Granting that this view is essentially correct, the excitatory process is then propagated from this area by muscle tissue to all parts of the heart. If the rate of propagation through auricular muscle is the same throughout, it may be expected that parts at a sufficiently greater distance from the sino-auricular node than nearby parts, would show an appreciable time difference in the onset of their contraction. Thus the left auricle, though in direct and common muscular connection with the right auricle, may be expected to contract later than the right. It is generally taught, however, that the contractions of the two auricles are synchronous.

Yet Chauveau (5), in 1894, stated that "in the horse, the right auricle begins its systole an appreciable time before that of the left auricle." Later Fredericq (6) took up the question and demonstrated the same fact in the dog's heart. Fredericq's pupils—Schmidt-Nielsen (7), Stassen (8)—confirmed his findings. The correctness of this statement has, however, been disputed by Hirschfelder and Eyster (9) who attribute Fredericq's results to the use of excised hearts; they believe that the heart in situ maintained under normal conditions, does not show any time interval in its auricular contractions and that such time interval occurs only in dying hearts or in hearts suffering in their nutrition. The later work of Fredericq and his pupils, was done on hearts in situ. Hirschfelder and Eyster's failure to demonstrate any time interval between the two auricular contractions may have been due to the use of a too slowly moving kymograph and of too small animals. Leontowitsch (10) likewise disagrees with Fredericq and finds that contractions of the two auricular appendages as well as of the atria may occur in various sequences, although in the majority of his experiments the right atrium and auricle contracted before the homonymous parts on the left side. Recently, Lewis, Meakins and White (11), tracing the course of the excitatory process through the auricles by the method of primary negativity, came to the conclusion that "certain portions of the right auricle contract before certain portions of the left auricle, and vice versa;" that it is therefore "unprofitable" to discuss whether it be the right or the left auricle that first contracts. While individual parts of the auricles doubtless begin their contraction at different times the point of interest insofar as the efficiency of the auricles as feed pumps is concerned, relates to the resultant of these differently timed contractions.[1] In view, therefore, of these contradictory findings it has been thought worth while to submit again the question to experiment by the usual graphic (suspension) method.

METHOD

Dogs of fairly large size—7.7 to 21 kgm.—were employed. They were anaesthetized with ether; no other drug was used. The vagus nerves were exposed in the neck and loose ligatures passed around them. The sternum was removed after ligation of the internal mammary arteries. The phrenic nerves were cut; the pericardium split open and its edges stitched to the sides of the opening in the thorax. Artificial respiration was started meanwhile and maintained throughout the experiment. The mixture of ether and air was kept in the neighborhood of 37°C. by means of Brodie's air warmer and anaesthetizer. The heart was prevented from drying by dropping upon it warm Ringer's solution. Cooling of the exposed heart was prevented by means of an electric lamp placed an appropriate distance above it.

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[1] It was realized early in the course of the experiments that the auricular myogram as obtained by the suspension method could not be depended upon to give an indication of the relative *duration* of right and left auricular systoles, still less any difference that might exist in the onset and duration of the period during which the blood is forced into the ventricles. Since then Wiggers (12) has published an interesting paper bearing on the relation between the auricular myogram and what he calls the "dynamic period" of the systole. While the relative *duration* of right and left auricular systoles may not be obtainable by the suspension method, the relative *time of onset* of the contractions is quite accessible to the method.

The tip of the right and left auricular appendages were connected to receiving tambours by means of François Franck pincettes. These tambours were covered with exceedingly thin and very elastic rubber dam (condom).

Rubber tubes of equal lengths joined the receiving to recording tambours provided with very light levers (thin straw and aluminum writing points). The recording tambours were placed upside down so that the curve of contraction is positive. An electro-magnetic tuning fork giving one hundred vibrations per second and a stimulation signal were mounted beneath the tambours. All writing points were adjusted to the same perpendicular line. The Hürthle kymograph was arranged for a speed of 180 mm. per second with the exception of experiment 1 where the speed was 320 mm. per second.

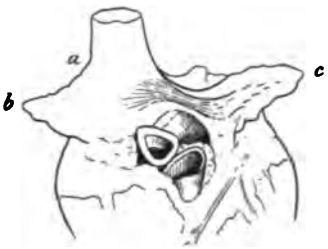

Fig. 1. Sketch of the dog's heart—ventral view—to show the position in which the auricular appendages were placed for the measurements mentioned in the text. The inter-auricular band is shown also.

At the conclusion of the experiment, the heart was removed and the following measurements were taken (*a-b*) distance from the position of the head of the S-A node (auriculo-caval angle) to the tip of the right auricular appendage; (*a-c*) distance from S-A node to the tip of the left auricular appendage (see fig. 1). Great care was taken in making these measurements not to stretch the heart tissue although it was necessary to raise the appendages; even with the utmost care, such measurements are liable to error and must therefore be considered as merely approximate.

RESULTS

The beginning only of each experiment will be discussed in this paper. The study of the effect of vagus stimulation has not been completed and its report must be postponed. By confining the observations on the inter-auricular interval to the beginning of the experiment, the criticism of cooling of the exposed heart and interference with nutrition through prolonged etherization, must be largely overcome. I may say, however, that usually, later phases of the experiment show but insignificant prolongation of the inter-auricular time interval.

A reference to table 1 shows that the average inter-auricular time interval in twelve dogs ranging in weight from 7.7 to 21.36 kilos was 0.013 second (fig. 2). This, therefore, corroborates the findings of Fredericq and his pupils. Moreover, the experiments which form the basis of this report have been remarkably uniform throughout; no such variations in the sequence of contraction as have been mentioned by Schmidt-Nielsen (loc. cit.) and by Leontowitsch (loc. cit.) were observed at any time.

TABLE I

NUMBER OF EXPERIMENT AND DATE	WEIGHT	RATE OF HEART BEAT	INTER-AURICULAR TIME INTERVAL	NUMBER OF MEASUREMENTS	A. DISTANCE FROM AURICULO-CAVAL ANGLE TO TIP OF RIGHT AURIC. APPENDAGE.	B. DISTANCE FROM AURICULO-CAVAL ANGLE TO TIP OF LEFT AURIC. APPENDAGE.	B-A
	kgms.		*seconds*		mm.	mm.	mm.
1. May 27, 1915.......	19.6	159	0.0130	34	29	54	25
2. June 25, 1915.......	15.7	285	0.0103	67	24	48	24
3. July 3, 1915.........	12.0	136	0.0142	15	28	45	17
4. July 30, 1915........	10.1	276	0.0105	14	27	42	15
5. August 5, 1915......	13.7	237	0.0102	12	25	50	25
6. January 4, 1916.....	8.45	175	0.0133	24	22	53	31
7. January 6, 1916.....	10.7	158	0.0132	20	30	60	30
8. January 19, 1916....	11.3	203	0.0100	32	29	55	26
9. January 21, 1916....	7.7	220	0.0100	11	29	44	15
10. January 24, 1916....	8.6	172	0.0137	11	23	40	17
11. February 2, 1916....	12.8	182	0.0169	32	32	52	20
12. February 9, 1916....	21.366	145	0.0165	60	41	76	35
Averages.............			0.013		28.2	51.5	23.3

It may be of interest to note in connection with the figure just given as the average inter-auricular time interval, that practically the same figure can be obtained from one of Lewis' tables (11) dealing with conduction time from S-A node to various points on the auricles. The conduction time from S-A node to the right appendage in 11 dogs was 0.0314 second; the distance was 28 mm. The conduction time from the node to the left appendage in 7 dogs was 0.0446 second; the distance was 44.6 mm. The difference in conduction time from the S-A node to the right and left auricular appendages respectively is therefore 0.0132 second, a figure which is practically identical with the one given here. It is difficult to state what significance, if any, attaches to this similarity in time differences. The contractions recorded in the manner described in this paper, doubtless give a record of the action of the greater mass of the auricle whereas Lewis' work was confined to a determination of the time of onset of excitation of a very limited part of auricular muscle relatively to the S-A node. If, as we must believe, the contraction of the auricle as a whole is the resultant of the differently timed contractions of its parts, the inter-auricular time interval obtained by recording the auricular contraction should be less than that obtained with the electrocardiograph, the contact points being S-A node and tip of appendages. The results presented in this paper may be so interpreted if reliance may be placed upon the measurements of the heart, for while in Lewis' animals the average difference in distance to be traversed by the excitatory wave was 16.6 mm., the difference in distance in our animals was 23.3 mm.

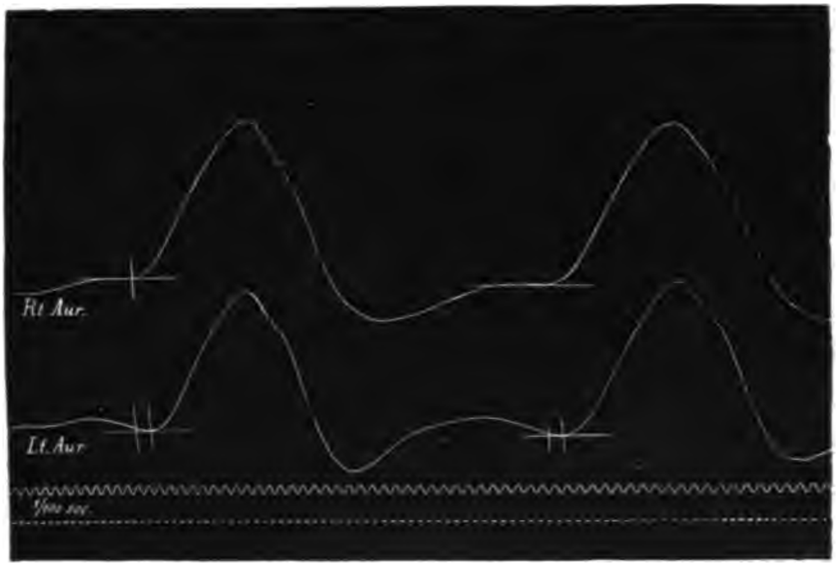

Fig. 2. Tracing of right and left auricular contractions obtained from dog 1.

It occurred to the writer that in view of the solidarity of the auricles, a special structure might exist whose special function would be that of inter-auricular conduction. An examination of the heart shows that a distinct band of muscle tissue stretches from the right auricle to the base of the left auricular appendage and forms apparently the most direct connection between the auricles. The same observation was made by Lewis, Meakins and White (loc. cit.) who call attention to the straight course taken by the fibers of this muscle band. This band has appropriately been called the inter-auricular band.

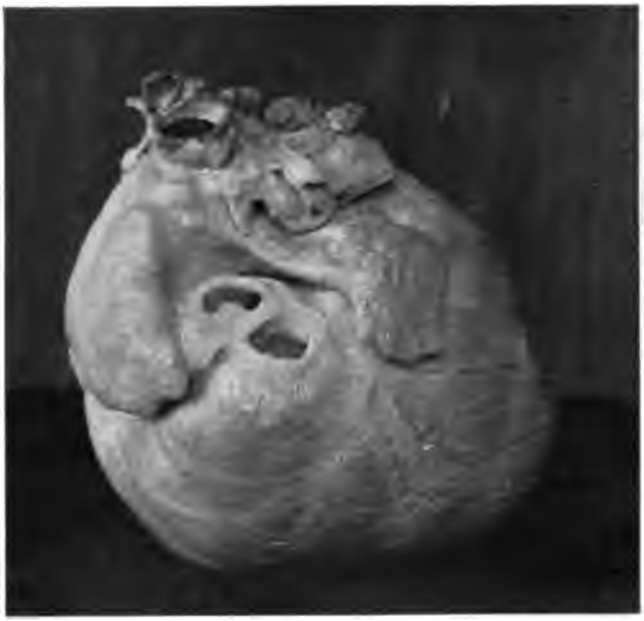

Fig. 3. Dog's heart hardened in the diastolic state to show the relations of the inter-auricular band. Natural size.

When the heart is hardened with 10 per cent formalin under a pressure of 30 cm. of the solution, the form and relations of the inter-auricular band are well brought out (see fig. 3).

The inter-auricular band. In a dog's heart of average size the right extremity of the band originates about 5 mm. to the mesial side of the auriculo-caval angle and in a direct line with it. This extremity of the band is immediately back of the aorta and spreads in a fan-like manner onto the mesial (right) auricular wall and the root of the superior vena cava. From this origin the band sweeps in an almost straight line to the base of the left auricular appendage but, so far as superficial appearance indicates, its fibers can be traced to near the tip of the left appendage. The band is thinnest near its origin on the right side where it has an angular appearance. It then broadens gradually as it passes to the left auricle. The band is slightly concave from right to left, the concavity being directed upward. It has a length of about 20 mm.

THE EFFECT OF CLAMPING THE INTER-AURICULAR BAND

If this band is of special importance in conduction its compression should lead to at least a lengthening of the inter-auricular time interval. A number of experiments were accordingly conducted in which the band was crushed about its middle between the jaws of a hemostatic forceps, care being taken not to injure the auricular tissue either above or below the band.

Extracts from the Protocols

January 4, 1916. Dog 6. After registering the auricular contractions for five minutes, an attempt was made to clamp the band by sliding a hemostatic forceps against the left wall of the pulmonary artery, the forceps being slightly inclined toward the right of the animal. The first attempt failed, a part of the auricular appendage near the base being compressed instead of the band proper. There was no apparent effect following this attempt. A second attempt succeeded. The inter-auricular time interval lengthened from an average of 0.0133 second to 0.98 second, many measurements showing an interval of 0.1 second (fig. 4). The inter-auricular time interval was therefore lengthened 73.68 times by the crushing experiment.

At the conclusion of the experiment examination of the heart showed, besides the compression at the base of the auricular appendage already mentioned, that the band was crushed transversely, the part crushed measuring 5 mm. The portion of uncompressed auricular tissue below the band measured 4 mm.; the portion above the band measured 10 mm.; these are external measurements and do not, of course, comprise any part of the auricular septum that may have been affected by the clamping.

January 6, 1916. Dog 7. In this and subsequent experiments the contractions of the left ventricle were registered simultaneously with those of the auricles. The first attempt to clamp the band was successful. The inter-auricular time interval passed from an average of 0.0132 second to 0.053 second an increase, therefore, of 4 times the normal average (fig. 5). The differently timed contractions of the two auricles were quite appreciable to the eye. The average rt.A–lt.V interval at the beginning of the experiment was 0.082 second; there was a gradual increase in this interval toward the close of the experiment, and it was 0.09 second just before clamping the inter-auricular band. This interval after clamping showed an average of 0.102 second.

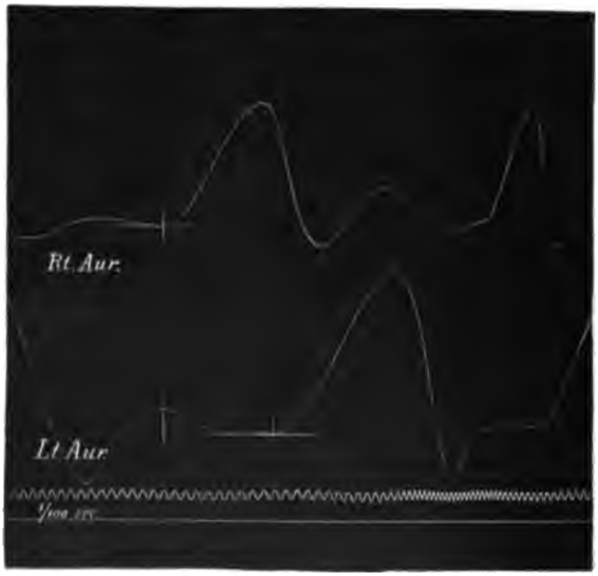

Fig. 4. Tracing from dog 6 showing the effect on the inter-auricular conduction time of injury to the inter-auricular band.

Examination of the heart showed that the forceps was applied on the band at about the same point as in the preceding experiment, this point being immediately to the left of the location of the auricular septum. The blades, however, included a little more of the tissue above and below the band. Viewed from the interior of the auricles there was no evidence of the compression on the right side of the auricular septum; but on the left side evidence of the compression was seen half-way between the opening of the right pulmonary vein and the opening leading into the left auricular appendage. The compression affected, in part, the roof of the auricle above the septum and, in part, the septal wall in front of the position of the fossa ovalis.

January 19, 1916. Dog 8. The inter-auricular time interval at the beginning of the experiment was 0.01 second; the heart rate 203 per minute. After section of the right vagus the heart rate rose gradually to 257 per minute; at the same time the inter-auricular time interval gradually decreased to 0.005 second. This was the time interval just before clamping. The clamp was applied in the same position as in dog 7; the inter-auricular time interval immediately after clamping and thereafter, had an average of 0.023 second an increase of 4.6 times the average observed before clamping. There was no appreciable change in the rt.A–lt.V interval, the average being 0.07 seconds.

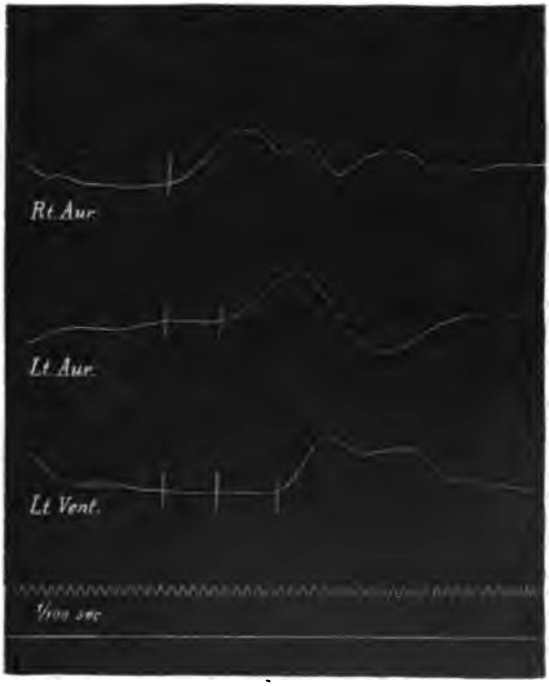

Fig. 5. Tracing from dog 7; it shows the usual effect of clamping the inter-auricular band.

The band was less well developed than usual; moreover, it had not been so well crushed by the forceps as the blades had failed to come completely together.

January 21, 1916. Dog 9. An attempt to clamp the band with Erlanger's clamp failed, the clamp being found imbedded in connective tissue on the side of the pulmonary artery. The experiment came to a premature close owing to an extensive tear in the left auricular appendage caused by an accidental pull on the pincette.

January 24, 1916. Dog 10. The band was clamped with Erlanger's clamp, but as it caused hemorrhage it had to be removed and the forceps applied. There was but slight increase in the inter-auricular time interval, viz., from 0.0137 second to 0.019 second—an increase of 1.38 times. The rt.A–lt.V interval had an average of 0.09 second. The records were obtained with difficulty owing to the restlessness of the animal (hemorrhage?).

The band and the auricular tissue immediately adjacent were found crushed by the forceps.

February 2, 1916. Dog 11. Immediately on clamping the band the inter-auricular time interval passed from 0.0169 second to 0.047 second—an increase of 2.8 times. The rt.A–lt.V interval maintained an average of 0.07 second throughout.

The band was found clamped a little to the left of the position described in the previous experiments, namely just above a connective tissue band which binds it to the base of the left ventricle (this connective tissue band is shown in figure 1).

February 9, 1916. Dog 12. Immediately after clamping the band the inter-auricular time interval passed from 0.0165 second to 0.052 second—an increase of 3.15 times. The rt.A–lt.V interval had an average of 0.07 sec. throughout.

The band was found clamped about its middle.

The results have been fairly uniform, in that, crushing of a portion of the band has invariably brought about a distinct interference with conduction as shown by the lengthening of the inter-auricular time interval from its average of 0.013 of a second to about 0.05 of a second. If we except the experiment on dog 10 in which there was considerable hemorrhage and the reading of the tracings was difficult, and the experiment on dog 6 where not only the band but also the base of the auricular appendage were crushed, it will be seen that the conduction time following injury to the band was from 3 to 4 times greater than the normal average.

The fact that the fibers of the band run a straight course from the region of the sino-auricular node to the left auricle and the recent findings of Lewis, Meakins and White (loc. cit.) that the highest rate of propagation of the excitatory process in the auricles is along this band (1252 mm. per second) offers an obvious explanation of the effect of injury to this structure. That it is the most direct and rapid route for the excitatory process is likewise demonstrated by comparing the effects of crushing the entire inter-auricular junction as described by Fredericq (12) with the effects of crushing the band alone as described here. Fredericq found that a complete crushing of the junction causes a complete dissociation of auricular action, the right auricle beating at its customary rate while the left auricle beats at a lower rate, viz., that of the idio-ventricular rhythm. The same phenomenon had been observed by Erlanger and Blackman (13) under different experimental conditions. When the compression is moderate the inter-auricular allorhythmia is temporary, each contraction of the right auricle is followed by a left auricular contraction but the inter-auricular time interval is considerably prolonged. In a tracing illustrating this condition the time interval is about 0.08 second. It would seem, therefore, that of the entire inter-auricular junction, the inter-auricular band constitutes the most important conduction path.

The clamping experiments here reported have demonstrated, I believe, the special importance of the inter-auricular band as a path of conduction between the auricles, for in all the experiments there was a considerable amount of uninjured tissue. This band, however, has not the same importance as a conducting medium as the auriculo-ventricular bundle for while, in the latter, crushing leads to a complete block, in the former, the same injury causes only a prolongation in the time of conduction.

SUMMARY

The time of onset of right and left auricular contraction, contrary to general belief, is not synchronous.

The excitatory wave originating in the sino-auricular node reaches the right auricle sooner than the left; hence the right auricle contracts an appreciable time before the left. The time difference averages 0.013 second.

The most important path of conduction between the two auricles appears to be the inter-auricular band. This special importance is

demonstrated by the effects of crushing the band, the conduction being delayed 3 to 4.6 times the normal average.

The inter-auricular band has not the same importance relatively to the auricles that is possessed by the auriculo-ventricular band, as crushing does not cause a complete block. Its importance lies in the circumstance that its fibers form the most direct inter-auricular path and that the rate of conduction is highest along this path.

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V. DIFFERENCES IN IRRITABILITY AND LATENT PERIOD IN DIFFERENT PARTS OF THE WALL OF THE STOMACH

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I have proposed in a recent paper to study the stomach from the point of view that it has been evolved from a simple tube much as the heart has been enlarged and specialized (1). Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of the primitive intestinal tube along the lesser curvature. These theoretical considerations led to the discovery that strips of muscle from different parts of the stomach wall vary markedly in their tendencies to rhythmic activity, in the rates of contraction, and in the forms of the curves. The most rhythmic region was found on the lesser curvature next to the cardia. The rate of contraction was generally faster and the rhythm more regular in the strips from the lesser curvature than in those from the greater curvature.

Work was done next to see whether differences could be found in irritability and latent period in different parts of the stomach wall, associated with the differences in contractility and based perhaps upon the same developmental peculiarities. Very little could be found in the literature bearing upon the subject. Meltzer (2) observed in dogs and rabbits that faradic stimulation of the outer surface of the stomach in situ gave different results in different regions. The fundus was quite unresponsive. Contractility seemed to improve as he approached the pylorus. Stimulation from the inside of the stomach had no effect except with the strongest currents. A local contraction of the small intestine could be obtained with a much weaker current than that needed for the stomach.

Lüderitz (3) hoped to explain the origin of gastric peristalsis by similar studies. Using mechanical, chemical and electrical stimuli, he noticed that the contraction often appeared cardially to the place stimulated. He thought there was a greater irritability near the lesser curvature than elsewhere. The reactions were very different in the pyloric antrum. When the stimulus was applied near the pylorus, there was a uniform contraction of the whole distal region. In dogs the fundus was quite insensitive to faradism.

Ducceschi (4) studied the irritability of different parts of the interior of the dog's stomach, using balloons with a device designed to apply the stimulus to the region distended. In the body of the stomach, the effects of mechanical stimulation were weak and slow to appear. He thought the reactions were quicker and more energetic at the pylorus than at the cardia. With the faradic current, the cardia proved to be the most irritable region, and its latent period was shortest. Such stimulation at the cardia sometimes caused inhibition when considerable activity was present. The fundus reacted very similarly but was much less irritable. The response of the pyloric antrum was even less than that of the fundus.

Weak acids had a more pronounced stimulating effect on the cardia than on the fundus. In the pyloric antrum, the effect was generally reversed, the active movements being inhibited. The presence of the balloon in the antrum almost always gave rise to active peristalsis. This was not the case in the fundus. When the antrum was contracting rhythmically, it was quite refractory to outside stimuli. This refractoriness of the active antrum was noticed by Rogers and Hardt (5) while studying the means of inhibiting hunger contractions. Carlson (6) has remarked that the movements of digestion are mainly in the pyloric part; the hunger contractions in the fundus. "Either these two regions of the stomach react differently to local stimulation of the gastric mucosa or else the nervous mechanisms concerned . . . . are different." May (7) found the inhibitory effect of vagus stimulation more pronounced on the cardiac than upon the pyloric end of the stomach.

A very suggestive paper is that of Barbera (8) who found that when the stomach of a frog was stimulated at any point by weak faradic shocks, the contraction appeared first at the cardia. By inserting a small manometer in the cardia, and by resting heart levers on different parts of the stomach, he found that this was due to a shorter latent period at the cardia. With the same strength of current a rise in the tube appeared 8.4 seconds after stimulating the cardia; 10.2 seconds after stimulating the middle region and 13.5 seconds after stimulating near the pylorus. In only one case, while stimulating the pyloric region, that part contracted first and then the cardia, followed by the middle. Ordinarily, stimulation anywhere on the stomach was picked up first at the cardia, and this insured the downward course of the peristaltic wave.

RESULT OF EXPERIMENTS

This work of Barbera on the frog seemed to me so important and fundamental that I have repeated it. Difficulties with the manometer method caused me to study the stomach in situ in pithed frogs, noting the time elapsed between stimulation and contraction by counting the seconds ticked off by a pendulum. The current was obtained from a Harvard Apparatus Company's inductorium (9) with one dry cell. Platinum electrodes were used, 3 mm. apart. The current was just perceptible to the tongue with the secondary coil at 12.5 cm. Stimulation was kept up for ten seconds, or until a contraction appeared. Care was taken to keep the preparation moist. The work was done in the months of September and October.

Although my results in ten frogs were not so uniform as those of Barbera, the cardia certainly gave by far the quickest reactions. There was generally less difference between the latent periods of the middle and pyloric regions than between those of cardia and middle region, and on four occasions the antrum reacted even more quickly than did the middle. This might have been caused by temporary changes in irritability due to previous stimulation or to the passage of spontaneous waves. The differences in latent period were brought out best by the weakest currents that would take effect in the antrum. Still weaker stimuli showed that the cardia was the most irritable part of the stomach, as it generally responded to currents that had no effect on the middle or on the antrum. Stimulation of the cardia was also more likely to cause a strong contraction of the whole stomach than was stimulation of the antrum. Frequently irritation of the pyloric region brought about contraction at the cardia and in the duodenum before any effect was observed at the point stimulated.

EXPERIMENTAL OBSERVATIONS ON THE STOMACHS OF RABBITS, CATS AND DOGS

A similar technic was followed with the stomachs of rabbits, cats and dogs. The rabbits were killed with a blow on the head; the other animals by etherization and bleeding. The stomach was removed quickly and placed in a shallow dish containing Ringer's solution at

38°C. The upper surface, while being tested, was kept moist by pouring on more warm Ringer's solution. Unfortunately, some cooling could not be avoided. The latent period in almost all of the experiments was determined visually, as has been described. Graphic records were obtained from a few stomachs, enough to show that the simpler technic was sufficiently accurate. These stomachs were suspended vertically and the region to be tested was picked up by two tiny serrefines; the lower one attached to a rigid support, the other to the writing lever. Such methods could not be used regularly on account of the time that would be consumed in making the fifty or more observations required on each stomach. It was feared that during this time there might be such changes in the irritability that no comparison could be made between the observations taken first and those taken last.

The results obtained on any single stomach would have served to illustrate what I wish to show, but it seemed best to average the observations made in the different regions. Figure 1 shows the location of these regions on the anterior surface of the rabbit's stomach, together with the average latent periods for faradic and galvanic stimulation obtained from 10 animals. Figure 2 gives similar data from the stomachs of 8 cats. The faradic current was obtained from the same apparatus that was used on the frog. The secondary coil was kept at 8 cm. in all the experiments. The galvanic current was just strong enough, 1.7 m.a., to duplicate the faradic effects in the cardiac and middle regions. The same platinum electrodes were used.

In the rabbit, the reaction was practically immediate in the esophagus and over the antrum cardiacum. It is well known that striated muscle reaches to the cardia in rabbits and dogs, but the articles consulted did not give the impression that it spreads over the stomach at all. Doctor Whipple kindly looked at some sections from these animals, and found strands extending 2 cm. beyond the cardiac thickening. This complication was not met with in the cat or man, where the lower fourth of the esophagus is free from striated muscle.

As will be seen from figure 1, the latent period was shortest around the cardia and on the lesser curvature as far as the incisura angularis. Beyond that, in the middle of the anterior surface, there is a zone in which the reactions appeared within a second or two. Outside of that again is an area, along the greater curvature, in which reactions were obtained in from one to four seconds in the more irritable stomachs (often from the younger animals). In some of the less irritable stom-

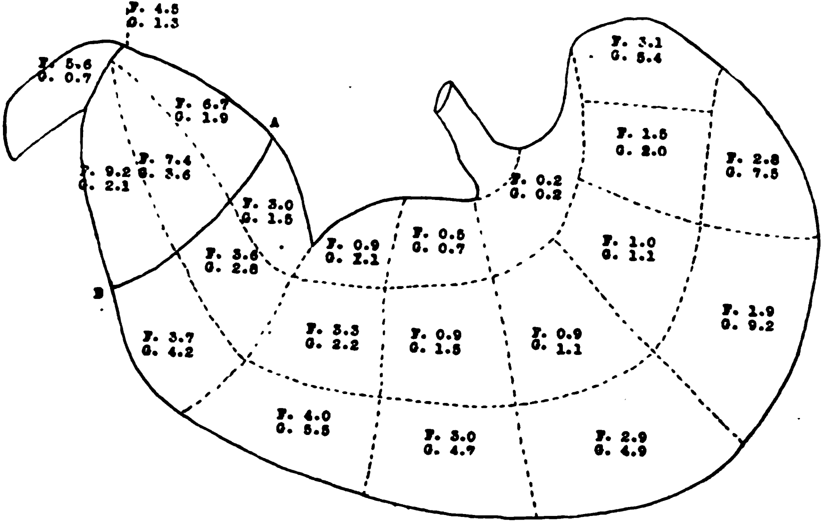

FIG. 1. Anterior surface of the stomach of the rabbit, showing the different regions stimulated, with the average latent periods after faradic and galvanic stimulation. The figures indicate seconds. *AB* is the dividing line between the pyloric antrum and the body of the stomach.

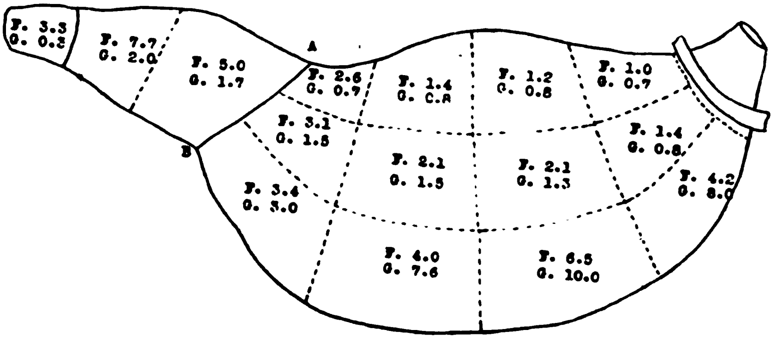

FIG. 2. Anterior surface of the stomach of the cat showing the different regions stimulated with the average latent periods after faradic and galvanic stimulation. The figures indicate seconds. *AB* is the dividing line between the pyloric antrum and the body of the stomach. The other unbroken line represents the pylorus.

achs, no reactions could be obtained at all with the strengths of current used. This comparative sluggishness of the greater curvature was more marked with the galvanic than with the faradic current. Experience showed that if no reaction could be seen after ten seconds, it was useless to stimulate further. The graphic records, however, suggested very strongly that such apparently negative results were more often due to the very small amplitude of the contraction rather than to an entire absence of response. It seemed best, therefore, in making the averages, to represent these results by 10 seconds, assuming that some reaction had occurred by that time. This has given results quite comparable to those found in any single stomach of moderate irritability.

Turning to the charts again, it will be seen that the latent period becomes longer as we approach the antrum pylori. Although the muscle begins to thicken in the preantrum, there is, in the rabbit, a very definite transition from the body of the stomach to the antrum. This can be brought out markedly by leaving the stomach 48 hours in ice-cold Ringer's solution. The dividing line is then very sharp between the gray-blue of the body of the stomach and the red muscle in the pyloric portion. This division is not so apparent in the cat and dog, but it can easily be demonstrated. In all the animals studied, the change in the type of reaction to stimuli was equally sharp at this line. In the preantrum there were deep hour-glass contractions; in the antrum, a centimeter away, there were peculiar local puckerings and tumefactions, often followed by a spasm of the whole pyloric end of the stomach. The latent period for the faradic current was often more than double that observed on the other side of the dividing line. Strange to say, there was no such increase with the galvanic current. This rather striking observation will be discussed later. The lesser curvature reacted a little more promptly than did the rest of the antrum.

It should be emphasized that the pyloric ring showed a shorter latent period than did the rest of the antrum. The duodenum, a few millimeters away, reacted to the galvanic current very promptly—within 0.5 to 1.0 second. For some unknown reason, the duodenum seemed to lose its irritability to the faradic current very rapidly after excision. With the organs in situ, both currents caused the duodenum to contract promptly.

POSTERIOR SURFACE OF THE STOMACH

It was soon noticed that in most stomachs the latent periods were a little longer on the posterior than on the anterior surface, and this conclusion was supported by the averages. This sluggishness was most marked along the greater curvature. The sequence of the four series of tests—with faradic and galvanic currents on both surfaces—was changed daily, although it was soon evident that the irritability of the stomachs remained quite constant for a period longer than the hour required to complete the work. To make sure that differences in thickness or resistance in the peritoneal coat could have nothing to do in altering the reactions, tests were made here and there before and after laying bare the muscle. No differences in latent period could be produced in this way.

EFFECTS OF MECHANICAL STIMULI

Experiments in which the excised stomach was pinched by fine mouse-tooth forceps gave results very similar to those with a weak faradic current. In the rabbit, the region about the greater curvature from the fundus to a point opposite the incisura angularis failed to react. The antrum showed the typical puckering contractions after a latent period of from 3 to 5 seconds. The pylorus and duodenum reacted almost immediately. The region around the cardia was so sensitive that a contraction generally appeared each time the Ringer's solution was poured on the stomach.

DIFFERENCES IN IRRITABILITY

With weaker currents, a close relation could be shown between the degree of irritability and the latent period. With one-fourth the strength of the galvanic current previously used, reactions were obtained (in the rabbit) only around the cardia; with one-half the strength, they appeared within a radius of 3 cm. from the junction of the esophagus and lesser curvature. This also was the only part of the stomach that reacted to the faradic current with the secondary coil at 13 cm. Similar results were obtained in the cat and dog.

Just as in the frog, stimulation, particularly of the middle region of the stomach, often showed itself first as a contraction at the cardia or along the lesser curvature. This might or might not be followed by a contraction at the point stimulated, depending on the strength and duration of the stimulus. This was very marked in the cat.

STUDIES WITH THE STOMACH IN SITU

To make sure that these differences were not due to the trauma of excision or to unequal post-mortem changes in the gastric plexuses, the work was repeated on the stomachs of rabbits, cats and dogs whose abdomens were opened after etherization. The vagi and splanchnics were left intact. The intestines were covered with gauze wrung out in warm Ringer's solution, and the stomach wall was moistened frequently with the same fluid. Regional differences were found corresponding to those in the excised stomachs. The cardia, lesser curvature and pyloric antrum reacted in about the usual time; but, strange to say, the rest of the stomach—the fundus and body—was generally much less irritable than in the excised organ, and often this region would not react at all to the currents used. The possible significance of these findings will be discussed later.

EXPERIMENTS ON THE HUMAN STOMACH

A few experiments performed on the human stomach, while necessarily inconclusive, suggest that similar differences will be found there also. Through the kindness of Doctors Baxter and Brill, I had the opportunity of making the desired tests twenty minutes after death in a man who died of nephritis. Unfortunately, there was ascites with edema of the peritoneum which may have had some influence on the reactions. Both in situ and when excised, this stomach was less irritable than those of animals, and to get reactions with the faradic current, the secondary coil had to be at 6 cm. The cardia and adjacent lesser curvature could not be studied satisfactorily in situ as the pulling that was necessary seemed to affect the reactions. Elsewhere the reactions were very much like those in the cat's stomach. After excision, the stomach contracted down to the cylindrical J form, so well known to radiologists. It seemed to lose its irritability rapidly: the preantral region was the only place which reacted twice in the same way, and no conclusions could be drawn.

Another specimen obtained at operation on a man with beginning malignant degeneration of a small scar at the pylorus showed definitely the difference between faradic and galvanic effects in the antrum; also the much greater irritability of the duodenum as compared with the stomach. Through the kindness of Dr. W. I. Terry, I was able to study the reactions in the pyloric half of another human stomach during an operation for an old cholecystitis. The anesthetic was gas-oxygen and the abdominal wound was infiltrated with novocain. The only definite statements that could be made after this work were that the stomach was less irritable than that of the animals; that it was a little more irritable near the lesser than along the greater curvature, and that the irritability of the duodenum was much greater than that of the stomach. Even with the coil at 4 cm., the faradic current failed to bring about a contraction in the stomach.

DISCUSSION

The lesser curvature, and particularly that part near the cardia, is more irritable than the fundus and body of the stomach; and it seems very probable that these differences can be ascribed to the developmental peculiarities outlined at the beginning of this paper. Other differences have been found, however, which show that the distance, spacially or embryologically, from the primitive tube is not the only factor modifying the irritability. As was suggested in the previous paper (10), the muscle of the stomach wall has probably been modified to serve different purposes in different regions. This would explain the big differences which I believe exist between the muscle of the body of the stomach and that of the pyloric antrum. Such differences seem inevitable when we remember the differences in function. While the principal activity of the fundus is to maintain a slight even pressure on its contents, the antrum must contract rhythmically and powerfully for hours at a time. Recently Lee, Guenther, and Meleney (11) have shown how remarkably the diaphragm differs from other muscles in its physiologic reactions and its chemical constitution. These differences are all related to its unceasing activity. For the same reason, we may expect some day to find that the reddish muscle in the pyloric antrum has more sarcoplasm, glycogen, etc., than the pale muscle in the fundus.

The better reaction of the pyloric antrum to the galvanic current suggests that the muscle is more sluggish in this region. One is reminded of the reaction of degeneration in striated muscle, or of such experiments as those of Fick (12) who noticed that an induced current which stimulated a frog's muscle had no effect on a mussel's muscle. The latter responded, however, to a slowly increasing galvanic current which had no effect on the frog. Further experience with excised strips has given me the impression that this sluggishness is due partly to nervous inhibition. If the irritability of the muscle is dependent upon its nervous connections, we should expect the reactions to become progressively slower after removal of the stomach from the animal and particularly after the trauma of cutting strips. The opposite is true: not only may the latent period become shorter, but it has been found in a number of instances that strips from the antrum would contract more promptly after they had been in the ice-box 24 and even 48 hours than they did immediately after excision. It has already been noted that the excised strips will generally beat rhythmically on the second or third day better than on the first. Although the enteric nerves are probably the last to die in the body (13), it is hardly conceivable that their functional capacity could *improve* after 48 hours, and we must look for the differences in the muscle itself.

The nervous inhibition with the stomach in situ probably explains Meltzer's failure to get good reactions from the cardiac half of the stomach. As will be shown in a subsequent paper, strips of muscle cut from such a refractory fundus and stimulated in a moist chamber will react markedly to the same current after a latent period of less than one second. Ducceschi has remarked upon the refractoriness of the pyloric region when it is active. It would appear that the nervous mechanism serves more to restrain the muscle than to raise its irritability. Such inhibition may be very necessary to save the stomach from the fatigue and perhaps incoordination that might follow its reaction to every stimulus received during digestion.

It is very probable that there are big differences again in the muscle of the duodenum, which not only contracts so differently, but has a shorter latent period and a greater irritability than that of the antral muscle. This was noticed by Meltzer. Schiff also remarked, years ago, that when the digestive tract is stimulated actively by the cutting off of its blood supply, the stomach, with the exception of the cardia, is the part least affected (14).

The tendency of the antrum to respond as a whole when stimulated at any one spot may have some clinical interest. Roentgenologists occasionally see such spasm in man when an ulcer is located in the pyloric region. As the pylorus is more irritable than the rest of the antrum, it is not surprising that it should often be closed so tightly when this part of the stomach is irritated. Interesting also to the clinician is the tendency to deep hour-glass contraction after stimulation in the preantral region in animals. That is the common location for such spasm in man. This finding is in agreement also with the observation that strips from this region show a far wider amplitude of rhythmic contraction than do those from any other part of the stomach wall.

I agree entirely with Meltzer that it is quite hopeless to attempt to influence the stomach therapeutically by intragastric or abdominal electricity. The refractoriness of the stomach in situ, particularly when stimulated through the mucous membrane, is so great that even powerful currents will produce no motor effect.

SUMMARY

It has been shown in the frog's stomach that the cardia is the most irritable region; also that the latent period is shortest at the cardia and longest near the pylorus. These peculiarities should have much to do with insuring the aboral course of the peristaltic waves.

The mammalian stomach has been studied from the point of view that it has developed from a primitive tube much as the heart has been enlarged and specialized. Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of this tube along the lesser curvature. These developmental differences probably have something to do with the regional differences in irritability and latent period found in the stomachs of rabbits, cats and dogs.

The latent period for faradic, galvanic and mechanical stimuli is shortest around the cardia and along the lesser curvature as far as the incisura angularis. These limits include also the most irritable part of the stomach. The region of the greater curvature and fundus is much less irritable and often fails to react at all.

There is, particularly in the rabbit, a definite transition from the pale muscle of the body of the stomach to the reddish muscle of the antrum. The change in the type of reaction to stimuli is equally sharp at this line. In the preantrum, there are deep hour glass contractions; in the antrum, a centimeter away, there are small puckerings often followed by spasm of the whole pyloric end. The latent period in the antrum is markedly lengthened for the faradic current; very slightly for the galvanic.

The pyloric ring is more irritable and reacts more promptly than does the rest of the antrum.

The duodenum is much more irritable than the pyloric antrum.

The posterior surface of the stomach is a little less irritable than the anterior surface, and the latent periods are longer.

With the exception of the cardia and lesser curvature, the stomach in situ is often quite refractory to stimulation in its cardiac half. This seems to be due to nervous inhibition as it is less marked in the excised stomach, and still less so in strips of muscle cut from the fundus or greater curvature.

It seems likely that the nervous mechanism serves more to restrain the muscle—to keep it from reacting to every stimulus—than to render it more irritable.

The little work done so far on the stomach of man has given results in agreement with those obtained in animals.

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THE VEGETARIAN DIET IN THE LIGHT OF OUR PRESENT KNOWLEDGE OF NUTRITION.[1]

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The prevalence of pellagra in the United States during recent years, and the accumulation of evidence that it is induced by a faulty diet, chiefly of plant origin, has aroused an interest never before equalled in the question of the adequacy of the strictly vegetarian diet. In the present paper we desire to discuss the vegetarian diet in the light of an extensive experience in feeding restricted diets to several species of animals.

Testimony as to the favorable results of human experience with what are purported to be strictly or practically vegetarian diets are so untrustworthy as to merit little confidence, because of the frequent consumption of foods prepared with milk, eggs, and butter, and the use of soups, gravies, etc., which include extracts of foods of animal origin. Practical experience furnishes abundant evidence that cattle, sheep, and other grazing animals grow from a comparatively early age entirely upon food derived from plants. We have previously reported considerable success in the nutrition of swine during growth on strictly vegetable mixtures when these contain the leafy portion of the plant (6, 12). We have not, however, found convincing evidence that any mammal has been adequately nourished from weaning time by a mixture of the seeds of plants and has made the maximum amount of growth and at the maximum possible rate. Pigs or rats may grow for a time on such rations, but always, so far as we are aware, at a rate slower than the normal, and they suffer suspension of growth before the normal adult size is reached. A colony of pigeons kept by the Department of Experimental Breeding at the University of Wisconsin has, however, lived several years in an enclosure and has been confined to a mixture of cracked corn, wheat, Canada peas, hemp seed, millet seed, and occasionally kaffir corn. They have been normal in every respect and their breeding powers have not suffered impairment. While pigeons in the open may vary their diet with insects, worms, etc., it seems hardly probable that this could possibly have happened to an appreciable extent in these caged birds. It would appear that the pigeon is not strictly comparable with pigs and rats as to its nutritive requirements.

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[1] Published with the permission of the Director of the Wisconsin Experiment Station.

The earliest well planned effort with which we are familiar to test the adequacy for growth and maintenance of mammals, of a vegetarian diet of wide variety, was made by Slonaker (1). He fed a group of rats on a strictly vegetarian mixture which included not less than twenty representatives of naturally occurring foodstuffs and afforded as great a variety as the human vegetarian in California would be likely to have on his table. Another group which served as controls received the same diet and in addition a small amount of meat two or three times a week. The diets were adhered to from the age of one month to the end of life and careful records were kept of the rate of growth and the degree of activity of all the animals. The vegetarian rats were frail and weak and showed extreme lassitude and indifference. The omnivorous controls were the reverse in all respects. They had an average span of life of 1020 days as compared with 555 days for the vegetarians. Slonaker concluded from a review of the literature relating to the subject on man and animals that similar results would be obtained if man were subjected to a strictly vegetarian regime throughout his lifetime.

Our own experiments in which rats were fed a wide variety of vegetable foods confirm in all respects the results of Slonaker. We did not attempt to keep our rats beyond the age of six months since our interest lay in their ability to make such a choice of foods as would best meet their nutritive requirements. The rats whose records of growth are shown in Chart 1, Lot 416 received corn, wheat, oats, rye, cooked beans, green peas, wheat embryo, corn gluten, and wheat gluten, flaxseed oil meal, green clover and green alfalfa, onions and peanuts.

Like Slonaker's rats they grew at about half the normal rate, but ceased to grow after the first ninety days. They appeared to be in a fairly good condition for a time thereafter, but were of course pigmies beside normal rats of the same age. One female even produced young, and did the best she could to suckle them, but at ninety days of age they weighed but 35, 39, 39, 61, and 36 grams respectively.

It is safe to say in the light of the results which were secured in this experiment as contrasted with those more recently obtained with certain simple vegetable mixtures in which no choice was permitted, (Charts 2, 3, 4,) that the instinct of the rat is not a safe guide to the selection of foodstuffs.

Voegtlin in 1914 (2) reported that rats, mice, and monkeys were unable to maintain their health for more than a brief period on a diet of corn, carrots, sweet potatoes, and oats. He observed, frequently in three or four days, lesions such as hyperemia and hemorrhage in the gastro intestinal canal. Sometimes the kidneys, lungs, and other organs showed congestion and slight hemorrhagic condition.

Latér studies by Koch and Voegtlin (3) have shown remarkable changes in the chemical composition of the nervous system of monkeys and rats fed exclusively on corn embryo which had been deprived of most of its fat by pressing. They state that the corn embryo is poor in substances preventing beri-beri. They have observed similar results when monkeys were fed corn meal alone, corn meal and sweet potatoes, or raw carrots as the sole source of nutriment.

Goldberger (4) has contributed further evidence tending to incriminate the vegetarian diet. Voegtlin, following the suggestion of Funk, expressed the belief that the production of pellagra is the effect of the deficiency of certain unidentified substances to which Funk gave the name vitamines, but he also suggests the presence of toxic substances such as aluminum in vegetable foods and a deficiency in the diet of certain amino acids as possible causes contributing to the production of the symptom complex seen in pellagra. Funk (5) believes in the existence of a number of vitamines, one preventing scurvy, another beri-beri, another pellagra, and has suggested the probability of the various species, e.g., birds and mammals requiring different vitamines.

Experiments conducted at the Wisconsin Experiment Station since 1906 and published in part in 1911 (6) revealed for the first time the evil effects for cattle of diets restricted to the wheat plant and the oat plant respectively, while the entire corn plant forms a perfectly adequate ration. The Experiment Station literature furnishes numerous reports of the failure of the corn kernel alone to properly nourish pigs during the growing period. Hart, Miller and McCollum have recently discussed the production of a condition in pigs which is histologically closely similar to if not identical with polyneuritis on diets containing a considerable variety of vegetable foods supplemented with minimal amounts of foodstuffs of animal origin (6). Holst (7) and his co-workers have reported the failure of guinea pigs to maintain life on oats as the sole food, whereas oats and cabbage proved adequate for the maintenance of well-being. This he attributed to a lack of an antiscorbutic substance which is supplied by the cabbage.

Young rats of 30 to 50 grams weight are unable to grow or to long maintain life when fed exclusively on one from the following list of vegetable products: Corn, wheat, oats, barley or rye kernel, polished rice, peas, beans, cottonseed meal, cottonseed flour, alfalfa, corn germ, wheat germ, flaxseed oil meal, peanuts, onions, cabbage, corn gluten, or wheat gluten. It is of great interest, therefore, to observe the effect of feeding a mixture of two of these ingredients, either of which alone fails to support growth or maintain well-being in the rat. Chart 2, Lot 478, shows how nearly nutrition approaches the normal expectation when rats are fed a simple mixture of alfalfa flour 40 and polished rice 60 per cent. Two of these rats, the male and one female, reached 83 and 80 per cent of the weight normal for the adult and are still in good condition after ten months on this diet. Two litters of young were produced by one of the females, but were not reared.

Chart 3, Lot 273, illustrates the practically complete nutrition of rats fed on a monotonous diet of ground corn (maize) 50, alfalfa flour 30 and peas 20 per cent. One female (6) has produced three litters of young during the first ten months on this diet and has successfully reared two litters. Her daughter, which never had any other food except that on which the mother had lived, grew to normal maturity (Chart 3, No. 7) and has brought forth a litter of young and successfully reared them, the young appearing perfectly nourished at the present age of twelve weeks. They have been confined to the same mixture which nourished their mother and grandmother.

Chart 4, Lot 271, illustrates the behavior of a group of rats fed exclusively from an early age (about one month) on a monotonous diet of ground corn 50, alfalfa flour 30, unextracted wheat embryo 20 per cent. Their growth was somewhat slower than the normal expectation, but three females have reached non-pregnant weights of two hundred grams, which is somewhat greater than that of many animals in our breeding stock which receive a mixture of corn 30, wheat 30, oats 30 and flaxseed oil meal 10 per cent, and a liberal supply of fresh milk daily, and clover or alfalfa daily when it can be obtained fresh.

The breeding record of this lot is poor, four females now nearing the end of their period of sexual activity, have produced but three litters, two of these litters being reared. The nutrition of these rats as shown by their appearance is faulty. At the age of one year they appear old and all are suffering somewhat with skin eruptions which cause scratching. Their hair is coarse and thin. Their ears and tails have remained free from inflammation and hemorrhage. These conditions we have frequently observed in rats fed upon certain rations of inadequate character. Cutaneous horns, often more than a centimeter long, frequently grow upon the noses of rats suffering mal-nutrition. These usually disappear within a month when the animals are placed upon a highly satisfactory diet. Certain dietary deficiencies lead to an inflamed and oedematous condition of the eyes (8).

An adequate working hypothesis concerning the essential factors which operate in the making of a successful food mixture is of fundamental importance as a guide to the study of the nature and extent of the deficiencies of the individual natural foodstuffs. Such a one is, we believe, now available. McCollum and Davis (9) working with diets consisting of purified protein, carbohydrate, fats, and salt mixtures have established the fact that in addition to these dietary constituents there must also be supplied two factors whose chemical natures are at present unknown.

Since, apparently, nothing is known regarding the chemical natures of these dietary essentials, and since the unknown factor which is present in certain fats seems to contain neither nitrogen nor phosphorus, we have for reasons set forth elsewhere (10) proposed to discontinue the term vitamine. This term is now frequently used to include a number of hypothetical substances each of which is supposed to so regulate the metabolism as to prevent a type of pathological functioning. Instead of using this general term to include all such substances, we have suggested the terms fat soluble A and water soluble B (10). The fat soluble A which is essential for growth and certainly in mammals for maintenance as well, was first observed in butter fat, but was later found to be present in many natural foodstuffs. Butter fat and egg yolk fat apparently contain more of it than any other natural foods. Animal tissues contain some of this unknown factor, especially the active organs. It is present in small, but wholly inadequate amounts in the cereal grains. A much greater content of it is found in the leaves of the forage plants, among which we have studied the flour prepared by grinding alfalfa leaves. The vegetable oils generally contain very little, if any, of this substance. It is a curious fact that certain seeds, such as the corn kernel which undoubtedly contains it, since rations so made up that the only factor lacking is supplied by butter fat, are also rendered capable of promoting growth to some extent in the absence of butter fat, by the inclusion of ground corn. Apparently in the plant cell this substance is not associated with the fats and is not readily removed by fat solvents. Possibly it is in chemical union and is in a form not soluble in fats.

The second dietary factor of unknown chemical composition, the water soluble B, appears never to be associated with the fat fraction as it is isolated from natural foods. It is readily soluble in water and in alcohol, but very slightly soluble in acetone, benzene, ethyl acetate and is insoluble in ether (10).

In our experimental diets we have employed either water extracts or alcoholic extracts of wheat embryo as a source of the water soluble B, since this material is so rich in it that two per cent of embryo in the diet is adequate for growth at the normal rate, provided all other factors are properly adjusted (9). For the fat soluble A, three to five per cent of butter fat suffices. Diets consisting of purified protein, dextrin and salts, which will not induce growth in young rats, likewise produce no growth when one of the additions, wheat embryo extract or butter fat is added, but becomes adequate when both are present (9).

Extracts which contain the dietary factor, the water soluble B, also relieve polyneuritis in pigeons which is induced by diets of polished rice or purified foodstuffs (10). There are many reasons for believing that it is one and the same substance which is concerned in the cure of this disease and in inducing normal maintenance and growth.

We question, for reasons which will appear later, the justification of Funk's assumption of the existence of several unknown chemical dietary constituents. We are convinced that all the observed pathological phenomena can be accounted for in maladjustments relating to the following factors:—the inorganic constituents; poor quality and inadequate quantity of the protein content; shortage of the substances A or B, or of both of them, and in certain cases the presence of toxic constituents in the natural foodstuffs. The latter may be of either inorganic or organic nature. Aluminum and colloidal silica in the one class have been suggested (2) and gossypol of the cottonseed, and the toxic effect of the oil of the wheat kernel which we have recently described (11) may be cited as examples of the second.

We have now tested fully on three natural foodstuffs viz. wheat, wheat embryo, and rice, our working hypothesis which postulates the need of but two chemically unidentified factors A and B as described above in addition to the well recognized constituents, protein, carbohydrate and inorganic salts. These studies make possible the interpretation of the ways in which the vegetable foodstuffs may give rise to metabolic disturbances.

The wheat kernel alone does not induce growth in the rat or the pig (12). By a systematic procedure we have fed to different groups of young rats the following rations: (1) Wheat and suitable salt additions which made the mineral content similar to that of milk; (2) wheat and a purified protein, casein; (3) wheat and butter fat to furnish the fat soluble A; (4) wheat, salts and protein; (5) wheat, salts and butter fat; (6) wheat, butter fat and protein; (7) wheat, salts, butter fat and protein.

It has been definitely established that while rations 1 to 3 inclusive improve the condition of the animals in some degree, appreciable growth or prolonged wellbeing is not possible. Chart 5, Lot 223, illustrates how ration (4) supports growth at the normal rate to nearly normal adult size followed by early failure. Chart 6, Lot 319, illustrates how ration (5) which includes the addition of both salts and butter fat to wheat fails to induce normal nutrition. These rats were very inactive, and this appeared to be the result of depressed sensibility. One was finally killed when in a very feeble state and one lung was found badly infected. The other slowly lost weight after reaching a weight of only 215 grams. Chart 7, Lot 380, shows relatively little growth on ration (6) when butter fat and protein were added to wheat. When failure was imminent and the animals exhibited partial baldness and eczematous condition of the skin, tail, and ears, a salt mixture of suitable composition was added, with the result that two of them promptly responded with rapid growth to the normal adult size and all made complete recovery from their pathological condition. Ration (7) (Chart 8, Lot 223 B) on the other hand is complete and induces growth to the usual adult size at the normal rate and supports reproduction at normal intervals and successful suckling of the young was the rule. These animals had sleek coats and appeared to be perfectly nourished. We have here a complete picture of the dietary deficiencies of the wheat kernel. In addition to proteins of poor quality, an inadequate content of the unknown substance, the fat soluble A, an unsatisfactory inorganic content, wheat also contains a toxic constituent which may contribute in certain rations to the failure of nutrition of the animals. This is brought out by the charts illustrating the cause of nutritive failure of rats fed wheat embryo.

Wheat germ, when fresh, is a fairly palatable food containing about 10 per cent of oil and 30 per cent of protein. In experiments with pigs confined in metabolism cages during forty days and fed a ration, the protein of which was derived entirely from this source, it has been found that as much as 39 per cent of the nitrogen ingested may be retained for growth. Similar experiments with the nitrogen of the entire wheat bread and with skim milk showed retention of 20 and 63 per cent respectively of the ingested nitrogen. The proteins of the wheat germ are, therefore, of good quality. This is borne out by the curves of Chart [illegible], Lot [illegible], in which the maximum growth took place on a ration containing but 10 per cent of total protein all derived from this embryo.

We have described elsewhere the complete series of experiments illustrating the nature of the dietary deficiencies of the wheat germ (11). Three of these are of especial interest in connection with the present discussion. On a ration of wheat embryo alone young rats do not grow at all. A ration consisting of fat free wheat embryo 33, dextrin 51, [illegible] 11, and butter fat 5 per cent induced growth at a rate [illegible] and maintained the animals in a good state for a [illegible] (Chart [illegible], Lot [illegible]). When the butter fat was omitted [illegible] the rate of growth was greatly depressed and [illegible] three months (Chart [illegible], Lot 521). When [illegible] additions were omitted from the ration of Lot [illegible] complete suspension of growth (Chart [illegible] [illegible] all animals responded [illegible] growth when the salt mixture employed was [illegible] similar to that of Chart [illegible] [illegible] employed (Chart [illegible]. The rats [illegible] the first four or [illegible] The important [illegible] of the wheat [illegible] of the animals [illegible] amount of the second unknown dietary constituent which we have described above as the water soluble B (Chart 14, Lot 401). The mixture of rice and purified additions just enumerated becomes a complete ration when there is added to it a small amount of a water or alcoholic extract of wheat embryo or of egg yolk. We now know that active extracts can be prepared from a long list of natural foods including unpolished rice, the cereal grains, vegetables and leaves of forage plants. Polished rice is comparable to a mixture of purified proteins, carbohydrates, and salt. Its protein content is probably too low to support maximum growth and it is poor in inorganic elements and these are probably not present in relations approximating the optimum. In addition, however, it is deficient in both the dietary factors A and B. In contrast to wheat which contains the B in abundance (See Chart 19, Lot 475), but requires three kinds of purified additions, rice requires four kinds to make it complete.

It has been pointed out that the ration of Chart 11, Lot 585, leads to failure because of its unsatisfactory inorganic content. When this is suitably modified by salt additions growth proceeds at the maximum rate (Chart 9, Lot 397). This serves as an illustration of how we can bring animals into a pathological condition and place the responsibility upon a single dietary factor. Chart 12, Lot 495, serves as an example in which nutritive failure follows as the result of the toxic constituent carried by the wheat germ, all other factors being known to be so adjusted as to induce maximum growth and apparently perfect nutrition for a long period. It is now possible to complete the series and describe rations which will lead to nutritive disaster, and to point definitely to each of the other dietary factors discussed above, as being singly responsible for the failure of the animals.

In Chart 13, Lot 324, the ration of polished rice, casein, dextrin, butter fat, a salt mixture and agar-agar, is entirely adequate for growth except that it lacks the water soluble B. Chart 14, Lot 401, illustrates the vigorous growth which ensued when this factor was supplied in the form of a cold water extract of wheat embryo. (Compare Charts 13 and 14). Chart 15, Lot 417, illustrates the failure of rats to grow on a ration of casein, dextrin, a salt mixture and agar-agar, the food mixture carrying an abundance of the water soluble B in its content of water extract of wheat embryo. The cause of this is the lack of the fat soluble A as is shown by Chart 16, Lot 418, which grew at the maximum rate when fed a ration closely similar in all respects, but containing 5 per cent of the butter fat.

Chart 6, Lot 319, shows failure of nutrition due to the low content of proteins of relatively poor quality. All the other factors in the ration are satisfactory and this ration becomes wholly adequate to support normal growth and reproduction when it is supplemented by a pure protein and no other addition (Chart 8, Lot 223B). Without protein addition the span of life was limited to nine to eleven months and the full adult size was never reached.

We are at present engaged in a histological and pathological study of animals whose failure of nutrition was brought about by the methods of feeding described above in which but a single dietary factor operated in inducing a pathological state. It is hoped that these results may materially advance our understanding of the effects of specific starvation.

Among the symptom complexes recognized by clinicians as attributable to inadequacy of diet are beri-beri, scurvy, and pellagra. The first two are now almost universally conceded to be due to a deficiency of a chemical factor in the diet. Hess (14) has reached the conclusion that the symptomatology of beri-beri and scurvy have many features in common.

Concerning pellagra disagreement still exists as to whether it is a dietary disease. The noteworthy achievements of Funk whose extensive investigations of avian polyneuritis entitle his views to respect believes that each of these diseases, beri-beri, scurvy, and pellagra is the specific result of a deficiency in the diet of a specific substance which he termed *vitamine* (15). Funk (5) apparently believes that the existence of three recognizable pathological states all attributable to faulty diet warrants the assumption of the existence of three unidentified chemical factors which form the specific vitamines, each of which when present so regulates the metabolism as to prevent a particular type of abnormal functioning of the cells.

The inadequacy of the sweeping explanation offered by Funk (16) and Voegtlin (17) that pellagra is caused by a lack of vitamines is apparent from Voegtlin's observations on animals fed corn meal and corn meal and sweet potato (3). Failure of nutrition with severe degenerative changes in the nervous system and early death were observed. We present in this connection the curves of growth of a group of rats whose ration consisted of ground corn 71.3, casein 18.0, agar 2.0, salts 3.7, and butter fat 5.0 per cent (Chart 17, Lot 568). The casein was purified to a practically ash free condition and must be regarded as free from vitamines as well. Aside from the essential dietary factor furnished by butter fat all chemical substances of unknown nature which could possibly be termed vitamines are supplied by the corn. These animals are in a state of excellent nutrition at the present time after six months on this diet. We do not wish at this time to discuss at length the nature of the dietary deficiencies of the corn kernel, but we can add that if in the above ration either the casein or the butter fat is omitted the nutrition of the animals is distinctly interfered with. Corn, according to our interpretation, contains a liberal supply of the water soluble B, but less than the optimum amount of the fat soluble A, together with proteins of poor quality, as one of us has already pointed out (18). Its inorganic content is entirely inadequate to support growth (Chart 18, Lot 618). The explanation for the failure of nutrition of the monkeys, of Koch and Voegtlin (3) is, we venture to suggest, to be found in a maladjustment of the well recognized chemical constituents of the diet rather than in "lack of vitamines."

Voegtlin (17) has recently discussed the effect of milling of corn on the removal of the vitamine-containing portion of the grain and has advanced the theory that the modern methods of milling of corn are an important factor in the causation of pellagra in the south. This is probably an important contributing factor, but there is certainly a great tendency to over estimate the importance of the as yet unidentified chemical factors lumped together as vitamines, and to attribute to them an importance paramount to the other factors of the diet. Although his experimental diets have all been entirely too complex to interpret, Goldberger (19) shows a creditable grasp of the situation when he states: "The possibility, if not the probability, of a 'twilight' zone within which a very slight change in any of the dietary components may cause an important shift of balance is not to be overlooked." We have in the present and in former papers described methods of experimentation which enable us to isolate the individual dietary factors and to evaluate in any natural foodstuff the individual dietary components, and have shown how the degree in which the shortcomings of one natural foodstuff are corrected by the supplementary character of another, can be studied by biological methods sufficiently sensitive to yield results of great practical value.

Our practically complete success in the nutrition of rats with strictly vegetarian diets made up of but three natural foodstuffs, and the failure attending the employment of a wider variety in the food mixture, emphasizes the fallacy of the assumption that the safest plan to insure perfect nutrition, is to include a wide variety in the selection of the constituents of the diet. So long as definite knowledge is wanting concerning the specific nutritive properties of the constituents of the diet, variety will unquestionably make for safety, but will not by any means assure safety, and indeed can scarcely secure the optimum result in any considerable per cent of cases. As soon as we possess an adequate knowledge of the specific properties of our natural foodstuffs and their supplementary relations to each other, it will certainly be possible to compound fairly simple and monotonous diets which can be depended upon to induce physiological well-being closely approximating the optimum.

The conscientious adherence to a vegetarian diet by one who has no adequate technical knowledge regarding the subject of diet appears to be fraught with danger since among the foods of vegetable origin, ordinarily consumed by human beings, several dietary factors are as a rule of an unsatisfactory chemical character. It is certain that all of the components of a successful diet are present in foods of plant origin.

The data which we have discussed in the present paper should we believe serve to orient investigations of this complex subject, and emphasize the great possibilities for human nutrition and animal production of properly directed research in this field.

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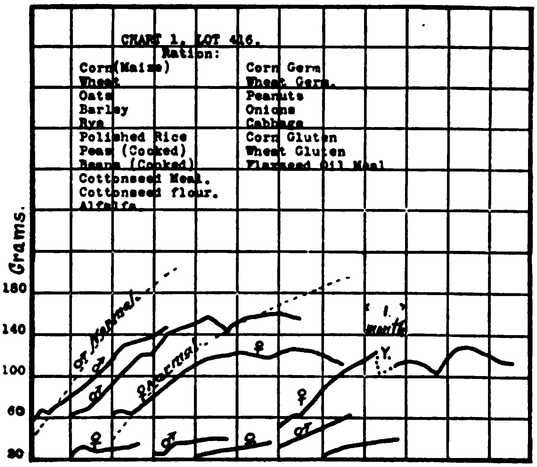
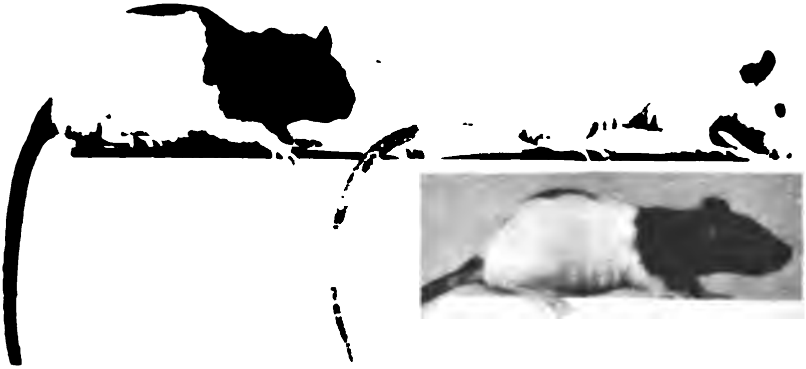


Chart 1. Lot 416 illustrates the failure of young rats to select from a liberal variety of vegetable foodstuffs the ingredients and proportions best suited to their physiological requirements. After 90 days there was no growth. One female even produced a litter of young and made an effort to rear them, but these young at the age of 90 days weighed but 35, 39, 39, 61, and 36 grams respectively. Everything necessary for complete nutrition was present in the foods regularly supplied, as is shown by the good growth of Chart 3, Lot 273, which received a monotonous mixture of corn, peas, and alfalfa flour. The cause of the poor nutrition of these animals is probably to be found in the relatively low content of proteins of poor quality, unsatisfactory relationships among the inorganic constituents of the diet, a low content of the fat soluble A and to some extent to the presence of toxic substances in certain vegetable foodstuffs. Appetite is not to be a safe-guide to the selection of food. The five short curves are those of the second generation.

In this and succeeding charts Y marks the birth of young.

This rat grew from weaning time to 83 per cent of the normal adult size on a simple mixture of polished rice 60, and alfalfa flour 40 per cent. Although somewhat undersized these rats appear nearly normal and a female has produced two litters of young after having grown up on this diet. (See legend to Chart 2, Lot 478.)

These rats were reared on a monotonous mixture of corn (maize) 50, alfalfa flour 30 and wheat germ 20 per cent. The albino is the mother of the hooded rat on the left. (See legend to Chart 4, Lot 271.)



The above photographs illustrate the appearance of three generations of rats which have been confined strictly to a diet of corn 50, alfalfa flour 30 and cooked peas 20 per cent. The mixture was finely ground so that the ingredients could not be picked out. The albino and her daughter are shown above, and her granddaughter below. (See legend to Chart 3, Lot 273.)

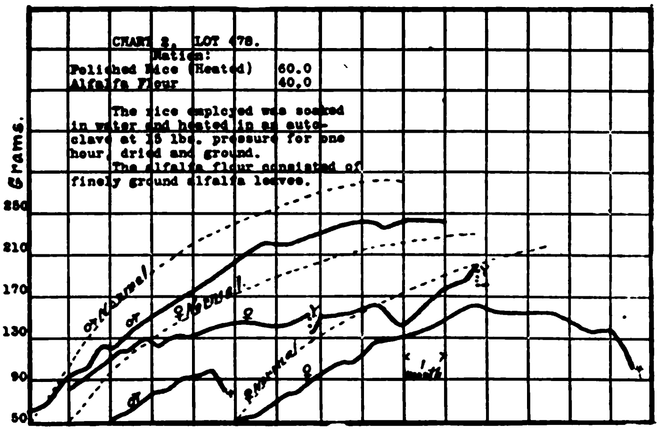


Chart 2. Lot 478 shows fairly good growth in the case of two animals to 80 and 83 per cent of the normal adult weight, on a monotonous mixture of polished rice 60 and alfalfa flour 40 per cent. These rats appear well nourished after nine months on this diet. It is evident that both the unidentified dietary factors, the fat soluble A and water soluble B are furnished by the alfalfa content of the ration, since heated polished rice contains neither of them. The cause of the failure of these rats to reach the full adult size rests principally in the low protein content of this ration, together with the relatively poor quality of the proteins. We are now studying the factors which contribute in this ration to prevent complete nutrition.

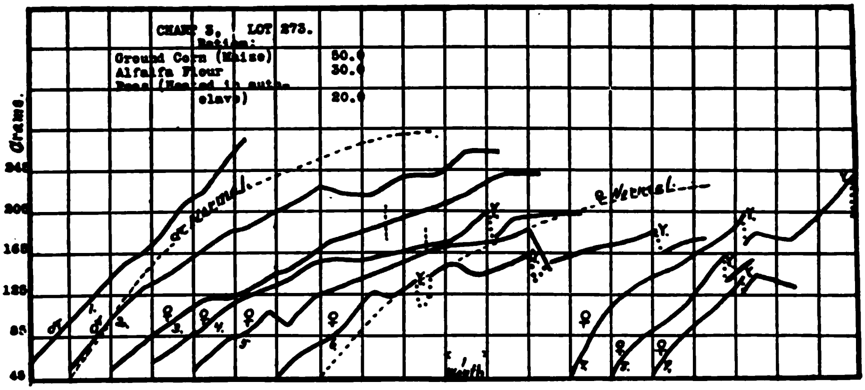


Chart 3. Lot 273 illustrates practically complete nutrition of the rat on a simple mixture entirely of vegetable origin. Rat No. 6 grew from the age of about one month on this diet of corn, alfalfa flour and peas, and produced three litters of young and two litters were successfully reared. Her daughter, No. 7, has been confined to the same diet all her life and has reached the normal adult size and has produced and successfully weaned a litter of young. These young appear perfectly well nourished and are of normal size for their age of twelve weeks.

The favorable results obtained with this ration are of special interest when considered together with the poor results of feeding a wide variety of vegetable foods, which included the three components of this ration. (Compare Chart 1, Lot 416.)

With an adequate knowledge of the dietary properties of each of the naturally occurring foodstuffs perfect nutrition can be attained in the omnivorous rat with a diet wholly of plant origin, but the animal cannot select with its appetite as a guide, the proper amounts and combinations which would best promote its well-being. Rats 3 and 4 were inferior breeders, and were isolated at the point marked on the curves and were given butter fat. There is no evidence that this improved their nutrition.

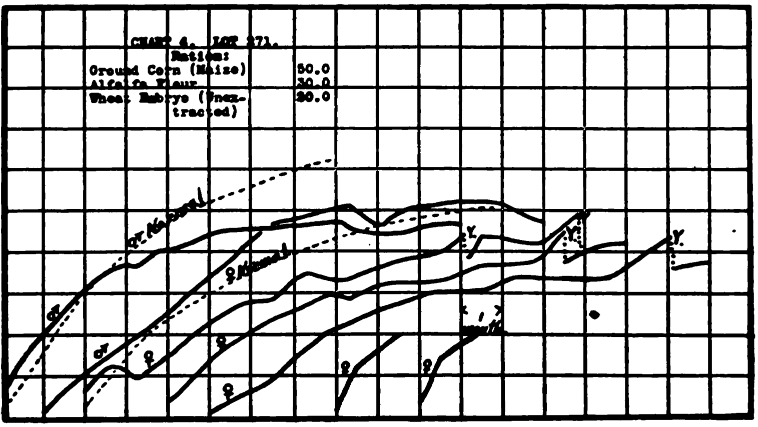


Chart 4. Lot 271 shows that wheat embryo when combined with corn and alfalfa flour does not nourish rats so successfully as does the same mixture with peas replacing the wheat embryo. (Compare Chart 3, Lot 273.) While the three females have reached the full adult size they have produced but three litters of young altogether, although they are now nearing the end of their reproductive period. The value of the protein mixture; the composition and content of the inorganic constituents; substances which act unfavorably on the animals, act together with a content of the unidentified essential dietary factors A and B, in determining the value of the diet. Without doubt palatability is frequently a factor contributing to success or failure.

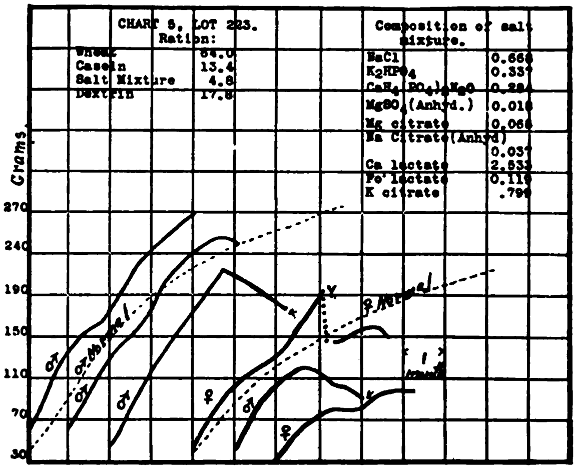


Chart 5. Lot 223 illustrates a diet which induces rapid growth for three to four months, but will not prevent rapid decline after that time. These rats presented a very miserable condition at the end of the period shown by their curves. The cause of failure is the lack of a single dietary essential, the fat soluble A. When this is supplied by the addition of butter fat the ration becomes entirely satisfactory for the completion of growth and for the normal amount of reproduction. (Compare Chart 8, Lot 223B.)

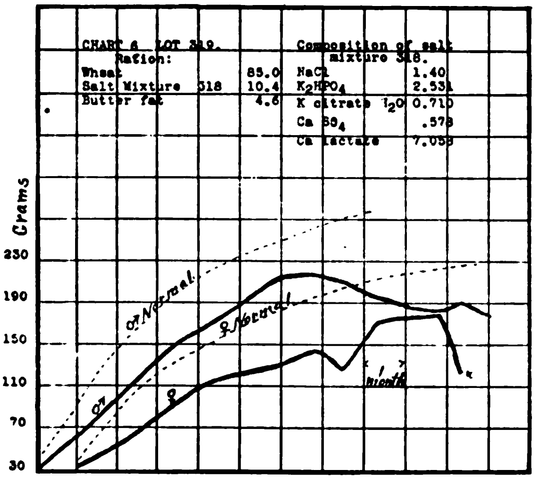


Chart 6. Lot 319 is another illustration of a ration which fails to support growth at the maximum rate and in which but a single dietary factor is responsible. (Compare Chart 5, Lot 223.) When pure protein (casein) is added to this food mixture, it becomes adequate for practically perfect nutrition of the rats. (Compare Chart 8. Lot 223B.)

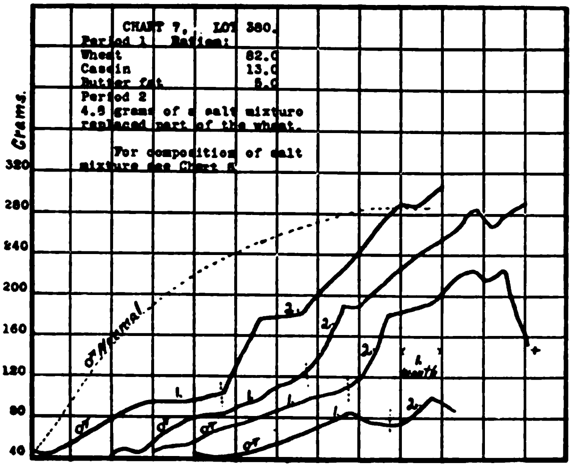


Chart 7. Lot 380 illustrates failure to grow normally on a diet which is adequate except in the character of its inorganic content. At the points marked on the curves a suitable salt mixture was added. The rats at this time had lost most of their hair and were suffering from an eczematous condition of the skin, ears, and tail. On the addition of the salt mixture there was a prompt acceleration of growth and a complete recovery from the pathological condition described. This ration with the salt mixture added is the same as that of Chart 8, Lot 223B.

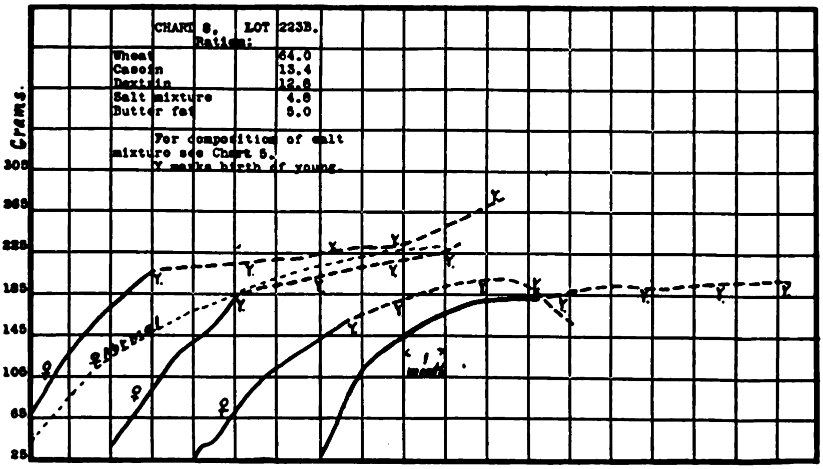


Chart 8. Lot 223B should be considered in connection with Chart 5, Lot 223, which lead to failure because of the lack of an adequate content of the fat soluble A, and with Chart 6, Lot 319, which led to failure because of a low protein content of poor quality. The above Chart (Chart 8) shows the complete nutrition of rats fed a closely similar ration with the above mentioned shortages made good. Although wheat contains a certain amount of a toxic constituent the ration of Lot 223B is otherwise so satisfactorily made up that the injurious effect of this is not apparent. (Compare Chart 9, Lot 397, Chart 10, Lot 521, Chart 11, Lot 585, and Chart 12, Lot 495.) This shows the fallacy of attempting to prove the presence or absence of an injurious character of moderate intensity in a food or drug by including it in a diet otherwise satisfactory. With a diet less efficient in one or more other factors the injury due to the toxic factor in question would become apparent.

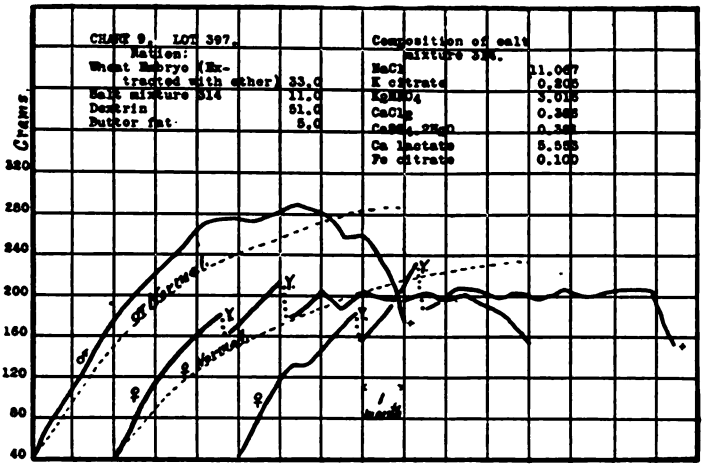


Chart 9. Lot 397 shows growth at the maximum rate on a ration of fat free wheat embryo, a salt mixture, dextrin and butter fat. Until they are nearly ten months old these rats appeared perfectly nourished. This Chart should be considered in comparison with Chart 10, Lot 521, which illustrates failure to grow normally on a ration like that of Lot 397 except that the butter fat as omitted, and with Chart 11, Lot 585, which was closely similar to that of Lot 397, except that in period 1 no salt additions were made. Chart 12, Lot 495, illustrates the injurious effect of wheat embryo which has not been freed from oil by extraction with ether. This series serves to emphasize that failure of nutrition may be induced by several factors other than by "lack of vitamines" an explanation which has in recent years seemed to satisfy both the uninitiated public and a considerable number of trained investigators.

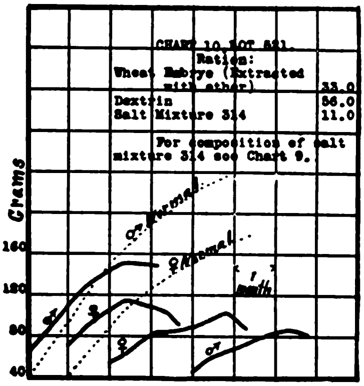


Chart 10. Lot 521. The ration of this group of rats was closely similar to that of Chart 9, Lot 397, except that the butter fat was omitted. Wheat embryo contains some of the fat soluble A, the factor supplied by butter fat, but without further additions of it this ration leads to suspension of growth and early failure. We have discussed elsewhere the dietary deficiencies of wheat embryo (11).

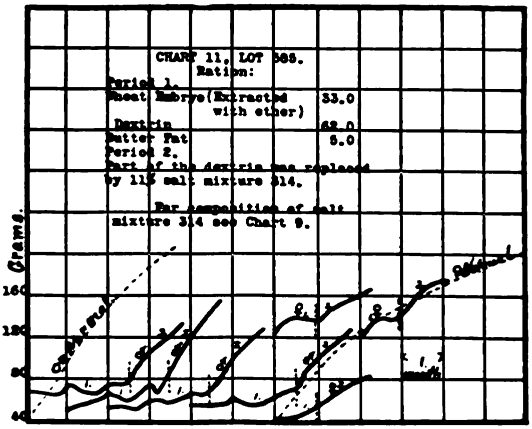


Chart 11. Lot 585 shows complete failure of rats to grow on a ration which differed from that of Chart 9, Lot 397, only in that the salt mixture was omitted. This serves as an illustration of a diet in which the omission or inclusion of a salt mixture determines complete failure or complete success in inducing growth.

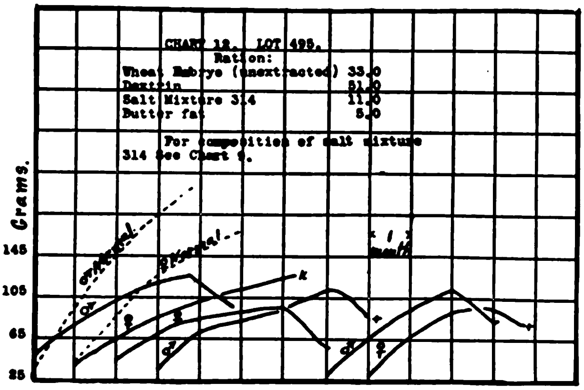


Chart 12. Lot 495 illustrates the marked injurious effect of the fats of the wheat embryo when these are included in the diet, as compared with a ration exactly similar except that the wheat embryo was extracted with ether. (Compare Chart 9, Lot 397.)

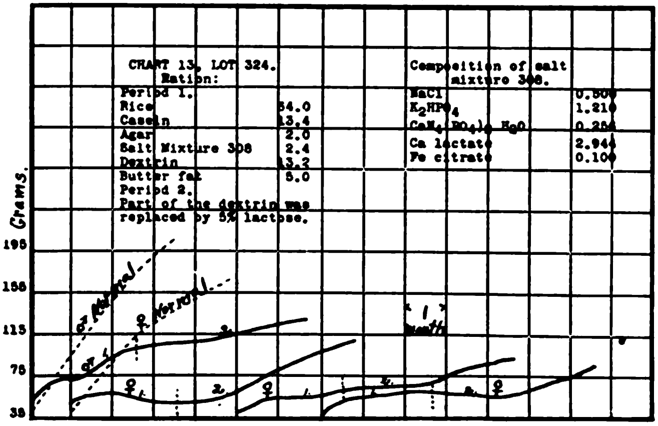


Chart 13. Lot 324 shows the failure of rats to grow on a food mixture which is lacking in but a single factor, the water soluble B. During period 2 a very small amount of this substance was added to the diet as an impurity of the 5 per cent of lactose which the diet contained. This Chart should be considered together with the records of Chart 14, Lot 401, whose diet was closely similar except that the water soluble B was added in the form of a water extract of wheat embryo.

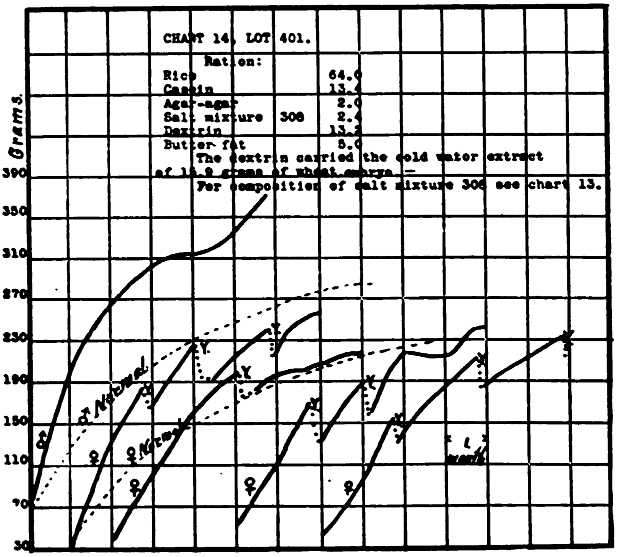


Chart 14. Lot 401, illustrates how the inclusion of the cold water extract of 15.9 grams of wheat embryo per hundred of ration transforms the food mixture from one which does not induce growth at all (Compare Chart 13, Lot 324, Period 1), to one which induced perfectly normal growth and repeated reproduction. The ration without the butter fat is again inadequate for growth. This is shown with a ration of somewhat different composition in Charts 15 and 16, Lots 417 and 418.

It is interesting to note, however, that of the nine litters of young produced by the four females in Lot 401 but two individuals were reared. The ability to reproduce and rear the young serves as a far more delicate test of the satisfactoriness of a diet than does growth (20).

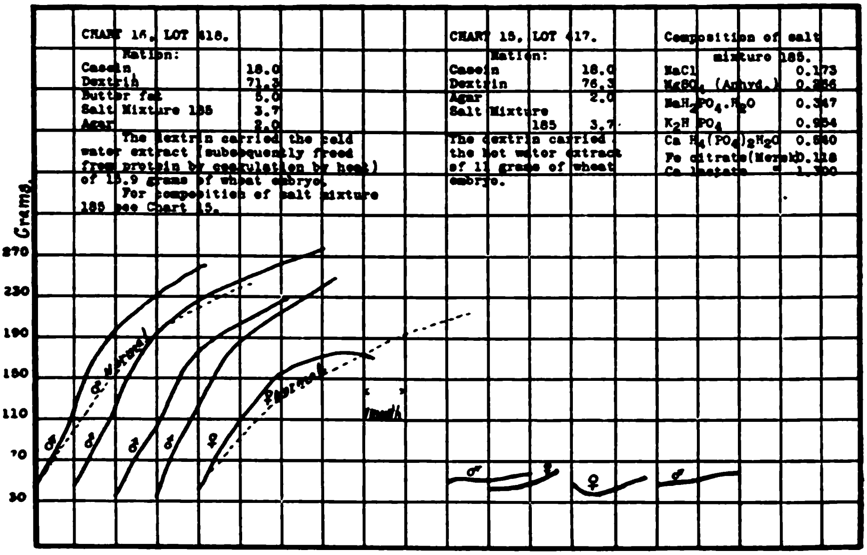


CHART 16, LOT 418.

Ration:

Casein	18.0
Dextrin	71.3
Butter fat	5.0
Salt Mixture 185	3.7
Agar	2.0

The dextrin carried the cold water extract (subsequently freed from protein by coagulation by heat) of 15.9 grams of wheat embryo.

For composition of salt mixture 185 see Chart 15.

CHART 15, LOT 417.

Ration:

Casein	18.0
Dextrin	76.3
Agar	2.0
Salt Mixture 185	3.7

The dextrin carried the hot water extract of 11 grams of wheat embryo.

Composition of salt mixture 185.

NaCl	0.173
$MgSO_4$ (Anhyd.)	0.266
$NaH_2PO_4 \cdot H_2O$	0.347
K_2HPO_4	0.954
$CaH_4(PO_4)_2H_2O$	0.540
Fe citrate (Merck)	0.118
Ca lactate "	1.300

Chart 15. Lot 417 illustrates the failure of rats to grow on a ration adequate in all respects except in a lack of the fat soluble A. When the latter is supplied in the form of butter fat the food mixture supports growth at the maximum possible rate. (Compare Chart 16, Lot 418.) The contention of Funk (15) that butter fat does not contain "vitamine" (i.e., a substance indispensable from the diet) has not as yet been supported by published experimental data, secured with diets so planned as to reveal the presence of what we have termed the fat soluble A.

Chart 16. Lot 418 should be compared with Chart 15, Lot 417. The latter fails to support growth because it does not contain the unidentified dietary factor, the fat soluble A. The former which is closely similar except that this factor is supplied by the 5 per cent of butter fat contained in the food mixture supports growth at the maximum rate.

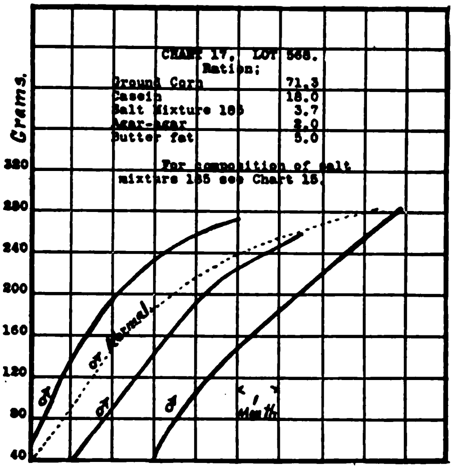


Chart 17. Lot 568 is included in the present series of illustrations to make clear the inadequacy of Voegtlin's assumption that pellagra is due to a deficiency of vitamines (17). All the chemically unidentified constituents of the diet except a part of the fat soluble A which is present in the butter fat, are supplied by the 71.3 per cent of corn in this food mixture. The failure of mice, rats, and monkeys to maintain a normal state of nutrition on a diet of corn (3) is due not to a lack of the unidentified constituents of the diet although corn is comparatively poor in the fat soluble A, but to a poor relationship among the inorganic constituents (Chart 18, Lot 618) and to its low protein content together with the poor quality of the latter. For growth in young rats the dietary A must also be increased.

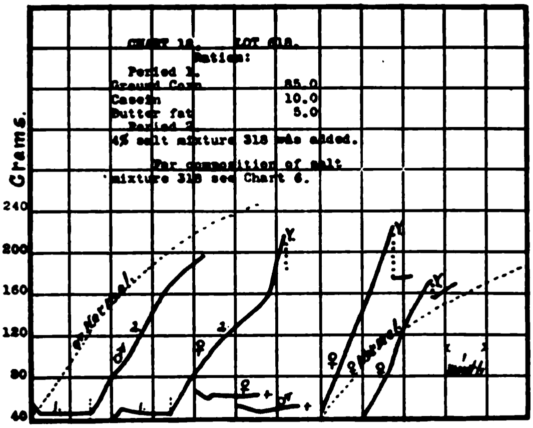


Chart 18. Lot 618 illustrates how inadequate is the character of the inorganic content of the corn kernel (maize) for growth. This ration is closely similar to that of Chart 17, Lot 568, except for a salt mixture addition in the latter. This, however, makes the difference between no growth at all and growth at the maximum possible rate. After complete suspension of growth for six weeks the addition of a salt mixture of suitable composition led to prompt resumption of growth at a rapid rate.

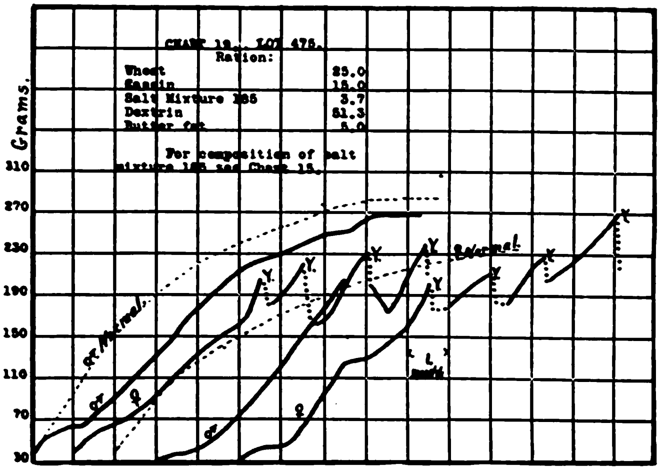


Chart 19. Lot 475 is included here to show the behavior of rats fed a ration deriving all of its water soluble B from its content of 25 per cent of wheat. This amount of wheat supplies enough of this factor to support normal growth and repeated reproduction, but does not suffice for the rearing of the young. Of the eight litters produced none were reared. This food mixture without the butter fat, is wholly inadequate to support normal nutrition in the rat over a prolonged period (Chart 5, Lot 223). While it is of course possible that there is contained in the extracts which we describe as furnishing the water soluble B more than one essential dietary factor, there is still wanting in the literature any experimental evidence in favor of the existence of more than a single indispensable chemical substance.

THE DISTRIBUTION IN PLANTS OF THE FAT SOLUBLE A, THE DIETARY ESSENTIAL OF BUTTER FAT[1]

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From the Laboratory of Agricultural Chemistry of the Wisconsin Experiment Station

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In 1913 it was pointed out in a paper from this laboratory (1) that certain fats of animal origin contained a substance the nature of which is as yet unknown, which is essential for growth or for long continued maintenance of health in the rat. Olive oil and lard were shown not to possess this growth factor. Later Osborne and Mendel described experiments which fully confirm our observations and added cod liver oil to the list of growth promoting fats, and almond oil to the list of fats which exert no special influence on growth (2). It was a matter of great importance to determine whether this indispensable dietary factor which we have termed the fat soluble A, (3) is present in foods of vegetable origin or whether it is a specific product of the mammary gland in mammals and the ovary in birds elaborated for the benefit of the suckling or of the embryo bird. We have for this reason examined various types of substances of plant origin, feeding these with rations so made up as to be wholly adequate when one of the growth promoting fats was included but inadequate when such fats were omitted. In the present paper we present the records of rats fed rations in which maize, cottonseed, linseed, olive, sunflowerseed, and soy bean oils respectively were the only source of the dietary factor supplied by butter fat.

All these have given results which make it clear that the fat soluble A is not found in fats and oils of plant origin so far as we have been able to discover. Owing to the importance of this fact, from the point of view of practical dietetics and animal production we desire to record these negative results for the more widely used plant oils.

One of the most interesting results of this investigation is the great difference in the effect on rats of consuming cottonseed oil which was prepared by extraction with ether, as compared with those fed a similar ration but with commercial bleached oil prepared by hot pressing. The former suffered distinct intoxication within a few weeks and showed rapid loss of weight (Chart 7, Lot 512) whereas the commercial sample could be fed at the same plane of intake with no signs of injury. (Chart 7-Lot 531). This result serves to corroberate the observations of Withers and Carruth (4), that the toxic factor "Gossypol" is soluble in ether and is present in the fat prepared by the use of this solvent.

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[1] Published with the permission of the Director of the Wisconsin Experiment Station.

Among the other oils of plant origin which we have studied, wheat oil, linseed oil and hydrogenated cotton seed oil have shown definite but not severe toxic effects on young rats. This we have been able to demonstrate by employing as rations, with which the fats were fed, food mixtures so made up as to just admit of about normal growth for several months but so low in their content of the fat soluble A as to render the animals sensitive to any unfavorable factor which might be superimposed on it in their delicately adjusted condition. In this way we have made it possible to test for toxicity of low intensity which would never induce visible effects if present in a diet which was otherwise of good quality.

This principle should be carefully considered by those who would study the harmfulness or innocuousness of various substances which may occur in the diet (5). Any of the several factors which make up the satisfactory diet can be singled out as the one to serve as the limiting factor to be so adjusted as to lower the resistance of the experimental animals.

In experiments in which the fat soluble A of the diet was supplied wholly by vegetable products it has been found that alfalfa leaves are an excellent source of this dietary factor, and that cabbage leaves also supply it. The cereal grains, while containing a small amount, are markedly inferior to alfalfa leaves in their content of this indispensable dietary factor.

The marked success which was reported by McCollum and Davis in restoring to health by the addition of corn, rats which had been brought to the threshold of death by a diet of supposedly approximately pure foodstuffs, should now be regarded in a new light because we have since learned of the tendency of lactose, which was a constituent of the earlier diets employed by us, to carry as impurities a sufficient amount of the unidentified dietary factors, fat soluble A and water soluble B, to supplement the content of these in any natural food, to a degree not to be ignored (6). Corn kernel fed with lactose led to improvement in the rats to a degree which corn alone could not induce (6).

In the preceding paper we have described rations in which all of the fat soluble A was derived from 40 per cent alfalfa leaves, and reproduction was observed after the rats had made all their growth and had continued ten months on the diet. Corn, wheat, oats and probably other grains supply this factor but in amounts below the requirement of animals over extended periods of growth.

The superiority of the forage portion of the plant over the seed with respect to its content of the fat soluble A is of considerable interest when viewed in the light of the dietary habits of lower animals. Those which consume the forage rations grow successfully from generation to generation on a strictly vegetarian diet, while the seed eating animals, so far as we have been able to learn, normally vary their diet to a considerable degree by the addition of green leaves, worms, insects, etc. Certain combinations of seeds may however suffice for normal nutrition.

An observation of interest in the experiments reported here, is that the ether extracted residue of corn meal is more effective in causing a slow but long maintained upward trend of the curve of growth than is corn oil. Compare Chart 10, Lot 451, and Chart 3, Lot 630. The interesting point is that ether extraction of plant tissue does not remove the substances essential for growth which is contained in butter fat. The obvious working hypothesis must for the present assume that the fat soluble A is in chemical union in the plant tissues, and in a complex which is not soluble in fat or in ether. In digestion and absorption it is set free and, being readily soluble in fats, thereafter accompanies the fats in the animal body. Owing to the large content of waxes, etc., extracted from plant leaves we have not been very successful in feeding ether extracts from these sources.

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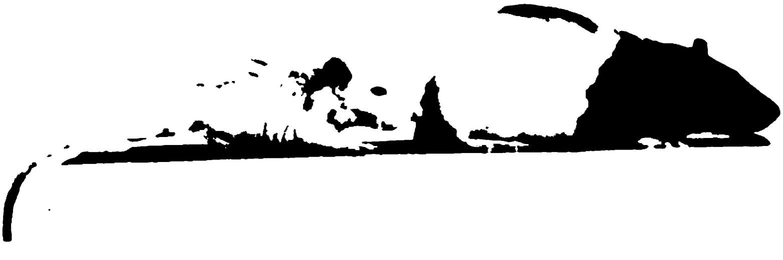
(2) Osborne and Mendel: Journ. Biol. Chem., 1913, xvi, 423.
(3) McCollum and Kennedy: Journ. Biol. Chem. 1916, xxiv, 491.
(4) Withers and Carruth: Journ. Agric. Research, 1915, v, 261.
(5) Carlson, Hektoen and LeCount: Journ. Amer. Chem. Society, 1916, xxxviii, 930.
(6) McCollum and Davis: Journ. Biol. Chem., 1915, xxi, 179.
(7) McCollum, Simmonds and Pitz: Journ. Biol. Chem., 1916, xxv, 105.
(8) McCollum and Davis: Journ. Biol. Chem. 1915, xxi, 638 and 642.

The rat on the right hand side, a male from Lot 441, received a ration exactly like Chart I, Lot 610, page 365, except that it contained 1.5 per cent of butter fat.

The rat on the left, Lot 447, received the same ration as Lot 441 except that 5 per cent of bleached cottonseed oil replaced the 1.5 per cent of butter fat and 3.5 per cent of rice.

The special property of butter fat in contrast to the plant oil as a source of the fat soluble A is most striking.



The rat on the right, a male from Lot 441, received a ration like Chart I, Lot 610, except that it contained 1.05 per cent of butter fat.

The rat on the left, Chart 2, Lot 497, received the same ration except that 5 per cent of sunflower seed oil prepared by ether extraction replaced the butter fat and 3.5 per cent of rice.

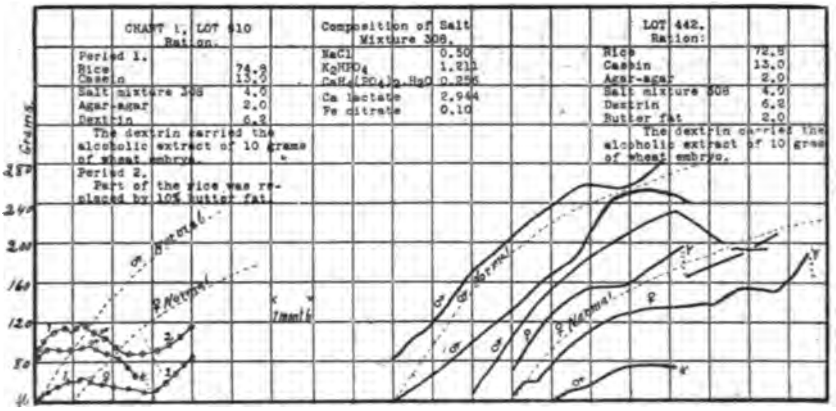


CHART 1, LOT 610
Ration.

Period 1.	
Rice	74.8
Casein	13.0
Salt mixture 308	4.0
Agar-agar	2.0
Dextrin	6.2

The dextrin carried the alcoholic extract of 10 grams of wheat embryo.

Period 2.
Part of the rice was replaced by 10% butter fat.

Composition of Salt Mixture 308.

NaCl	0.50
K_2HPO_4	1.210
$CaH_4(PO_4)_2.H_2O$	0.256
Ca lactate	2.944
Fe citrate	0.10

LOT 442.
Ration:

Rice	72.8
Casein	13.0
Agar-agar	2.0
Salt mixture 308	4.0
Dextrin	6.2
Butter fat	2.0

The dextrin carried the alcoholic extract of 10 grams of wheat embryo.

Chart 1. Lot 610 illustrates the behavior of rats fed a ration which is adequate in all respects except that it lacks one of the dietary essentials, the fat soluble A which is found in butter fat, egg yolk fat, ether extract of kidney, etc. We selected this diet as one to which the more important vegetable fats could be added in order to test for the distribution of this substance in fats of plant origin. The ration fed to this lot of rats becomes entirely adequate to support growth to maturity at the normal rate when butter fat is added. (See Chart 1, Lot 442.) There was a prompt response with growth in period 2 when butter fat was introduced.

Lot 442. The ration fed these rats differs essentially from that employed in Lot 610, Chart 1, only in containing 2 per cent of butter fat. With the butter fat present, growth is complete; without it no growth can take place. Any fat of plant origin which contains the dietary factor supplied by the butter fat, should when incorporated with this ration, induce growth.

In this and the succeeding charts Y marks the birth of young.

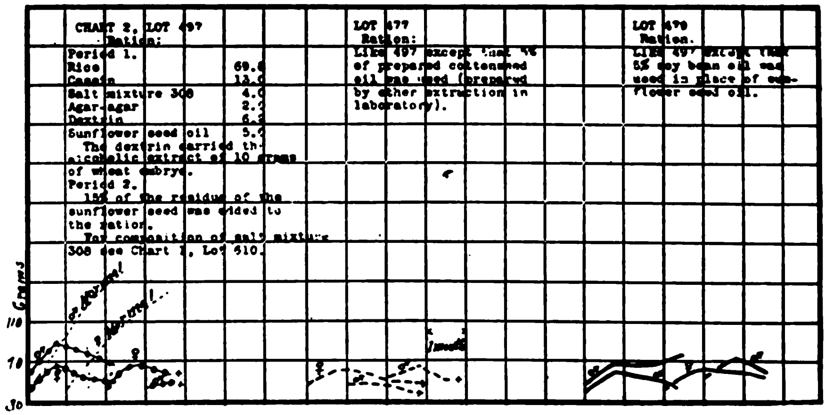


CHART 2, LOT 497
Ration:
Period 1.
Rice 69.
Casein 13.
Salt mixture 308 4.
Agar-agar 2.
Dextrin 6.
Sunflower seed oil 5.
The dextrin carried the alcoholic extract of 10 grams of wheat embryo.
Period 2.
15% of the residue of the sunflower seed was added to the ration.
For composition of salt mixture 308 see Chart 1, Lot 510.

LOT 477
Ration:
Like 497 except that 5% of prepared cottonseed oil was used (prepared by ether extraction in laboratory).

LOT 479
Ration.
Like 497 except that 5% soy bean oil was used in place of sunflower seed oil.

Chart 2. Lot 497 shows the failure of sunflower seed oil to promote growth when included in the ration employed in this series. With butter fat instead of sunflower seed oil normal growth would have resulted. (Compare Chart 1, Lot 442.)

Lot 477 likewise shows failure of rats fed 5 per cent of cotton seed oil prepared by ether extraction with a ration fully adequate to support growth except that it lacks the fat soluble A. These rats showed distinct signs of injury from the effects of eating even this small amount of cotton seed oil prepared by extraction in the laboratory, in contrast to commercial oil prepared by pressing and subsequent bleaching, The latter showed no toxic effects but failed to induce growth. (See Chart 4, Lot 447B.)

Lot 479 shows that soy bean oil does not have the growth promoting property possessed by certain fats of animal origin. (Compare Chart 1, Lot 442.)

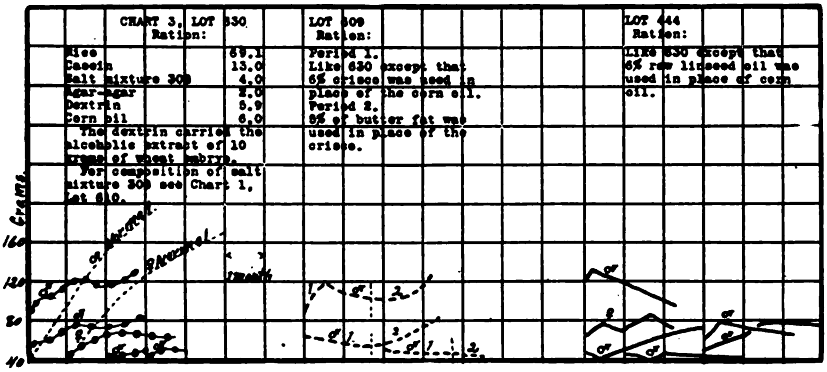


Chart 3. Lot 630 illustrates the inefficiency of corn oil to induce growth. The ration here employed is closely similar to that of Chart 1, Lot 442, except that 6 per cent of corn oil replaces 2 per cent of butter fat. The corn oil employed in this ration was prepared by ether extraction in the laboratory.

Lot 609 shows the failure of a widely used commercial substitute for lard, Crisco, made by hydrogenating cotton seed oil, to induce growth in young rats. On replacing the Crisco by butter fat in the second period growth proceeded.

Lot 444, shows that 6 per cent of linseed oil fails to induce growth. We have been unable to find a single instance where the isolated fats and oils of plant origin promote growth in the manner that certain animal fats do. The rats in this group appeared very rough coated, emaciated and feeble and were in a much worse condition than those of Chart 1, Lot 610, whose ration was closely similar but contained dextrin instead of linseed oil. Linseed oil is somewhat detrimental to the rat. This does not indicate the absence of the fat soluble A from plants. We have already pointed out that alfalfa, wheat germ, maize kernel and cabbage furnish a considerable amount of this substance.

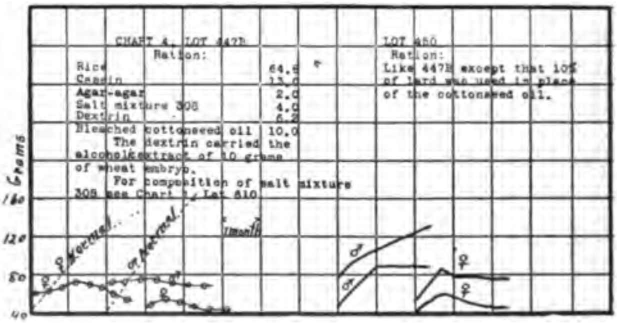


Chart 4. Lot 447B shows the absence of the dietary essential the fat soluble A from bleached commercial cotton seed oil. Even 10 per cent of the latter fails to induce appreciable growth.

Lot 450 serves to confirm our earlier observations on lard, which showed it to possess very little of the growth promoting properties of butter fat, i.e., the fat soluble A. In our early work the content of the water soluble B was dependent on its presence as an impurity in the lactose. In the present series of experiments an abundance of this factor, was added in the alcoholic extract of wheat embryo.

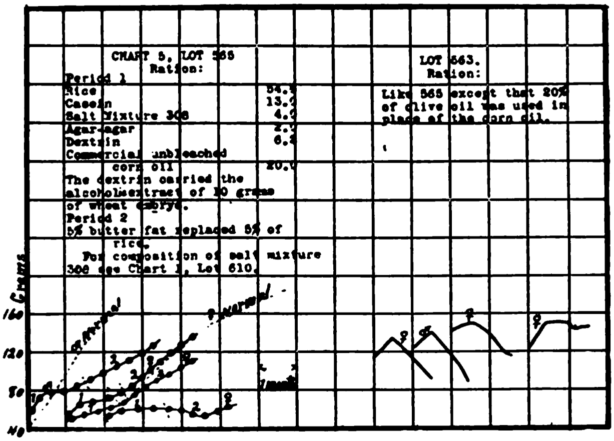


CHART 5, LOT 565
Ration:
Period 1
Rice 54.
Casein 13.
Salt Mixture 308 4.
Agar-agar 2.
Dextrin 6.
Commercial unbleached corn oil 20.0
The dextrin carried the alcoholic extract of 10 grams of wheat embryo.
Period 2
5% butter fat replaced 5% of rice.
For composition of salt mixture 308 see Chart 1, Lot 610.

LOT 563.
Ration:
Like 565 except that 20% of olive oil was used in place of the corn oil.

Chart 5. Lot 565. The rats in this group received 20 per cent of commercial unbleached corn oil. Even this amount does not suffice to furnish an adequate amount of the fat soluble A to induce growth.

Lot 563. In 1913 it was first shown in a paper from this laboratory that butter fat possessed a certain growth promoting property and that olive oil did not, (1) We later learned that the success of these early experiments turned upon the degree of purity of the lactose contained in the rations. The two dietary essentials, the fat soluble A and the water soluble B, at that time unappreciated, were carried by the lactose as impurities and were present in minimal amounts which could suffice to induce growth. Since pure lactose would cause failure to grow even when butter fat was included in the ration, it seemed desirable to repeat the test on olive oil with the rations known to be wholly adequate with respect to all factors except for the dietary A. The curves shown here confirm our earlier results in indicating the absence of the fat soluble A from olive oil.

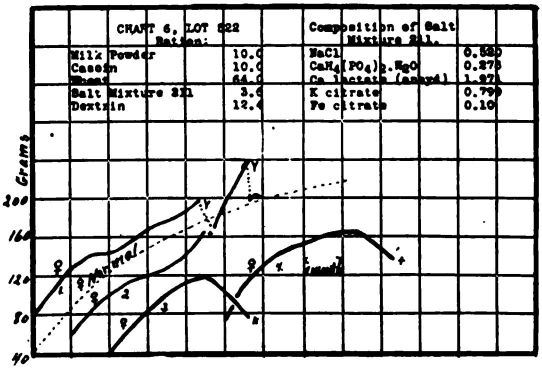


Chart 6. Lot 522, illustrates the curves of growth of four rats fed a ration which contained barely enough of the fat soluble A to induce growth at the normal rate during a period of three to five months. Number 1 produced two litters of young but did not rear any of them. After the loss of the second litter she did not appear to be in a healthy condition, so she was discarded. Number 2 died just before producing a litter of young. The remaining two suddenly declined and died after having grown fairly well for a time. With butter fat added (5 per cent) this is one of the most successful rations we have employed for inducing repeated reproduction and rearing of the young. (8)

This ration was therefore employed to test the growth promoting values of certain vegetable oils since a relatively small additional content of the fat soluble A should supplement the amount already in the ration and prevent failure. We look upon the experiments with this ration as somewhat more delicate tests for this dietary factor than were those with the ration described in Chart 1.

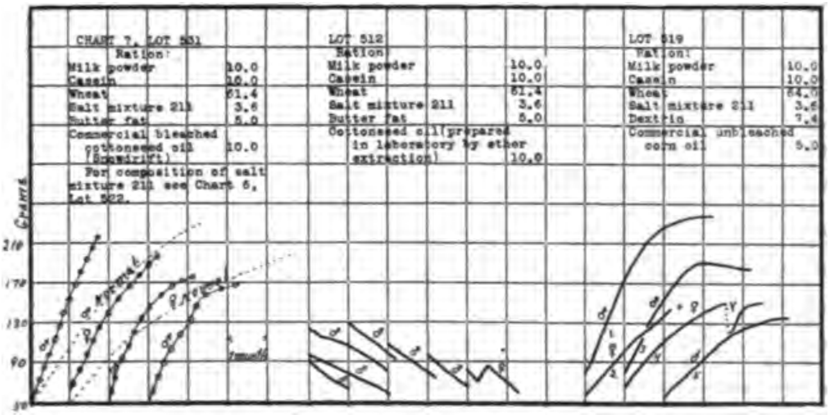


Chart 7. Lot 531. This lot was given the ration described in chart 6 but with the addition of butter fat together with 10 per cent of bleached commercial cotton seed oil to see whether the latter contained enough of a toxic constituent to interfere with growth. There is no evidence that this content of refined cottonseed oil was detrimental. According to the manufacturers the oil was prepared by pressing.

Lot 512, shows the injurious effect of feeding the same ration as was given Lot 531 but employing instead of refined cotton seed oil a preparation made by extracting cotton seed with ether in the laboratory. This result is in harmony with those of Withers and Carruth in showing the extraction of the toxic principle of cotton seed "gossypol" by ethyl ether. (4) (Compare Chart 2, Lot 477.)

Lot 519. Received a ration like that of Chart 6 Lot 522, except that 5 per cent of commercial unbleached corn oil was included instead of its equivalent of dextrin. There was no evidence of improvement as a result of the content of corn oil. Number 2 died in parturition, number 4 produced a litter of young but destroyed them.

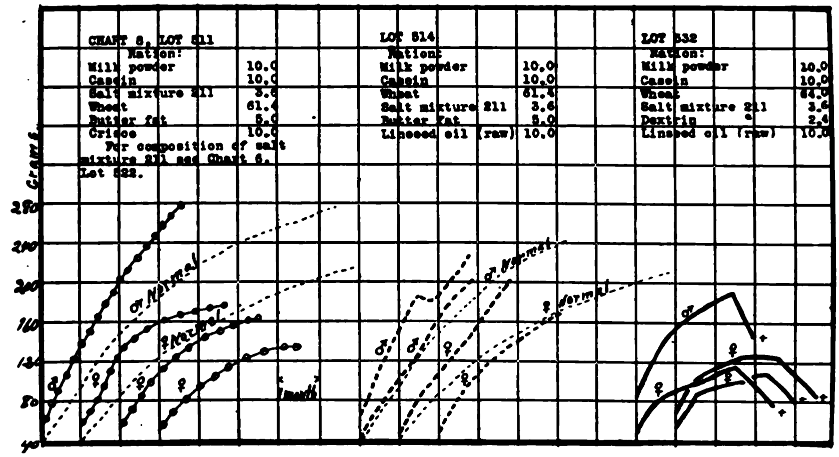


Chart 8. Lot 511. The ration of this lot was like that of Lot 522, Chart 6, but without butter fat added, and with 10 per cent of Crisco replacing an equivalent amount of dextrin. The appearance of these rats was distinctly inferior to that of Lot 522. Apparently the content of butter fat did not suffice to counteract a somewhat deleterious effect of the hydrogenated cotton seed oil. We have fed this food mixture with dextrin replacing the Crisco, and in all cases the animals were extremely sleek and well nourished (8 p. 642.) Lot 511 on the other hand suffered from roughness of the skin, tail and ears and appeared to be very poorly nourished.

Lot 514, received a ration like the preceding one but with 10 per cent of raw linseed oil replacing Crisco. There were no definite signs of any ill effects resulting from the consumption of linseed oil. Chart 3 (compare Lot 444). Linseed oil does cause injury in some degree however although its detrimental effects are not visible when the ration contains butter fat. When the butter fat was omitted from this food mixture the depressed growth and early failure of the rats was very pronounced as compared with the behavior of rats fed a similar ration but without the linseed oil. (Compare Lots 532 with 514 and Chart 6 Lot 522.)

Lot 532, shows that 10 per cent of raw linseed oil in this ration exerts a depressing effect on the growth and health of the animals. These rats became very emaciated and suffered from inflammation of the eyes. This ration with dextrin replacing the linseed oil is that feed to Lot 522, Chart 6. The bad effects of the linseed oil are not visible when the ration is improved by the inclusion of the butter fat as in Lot 514.

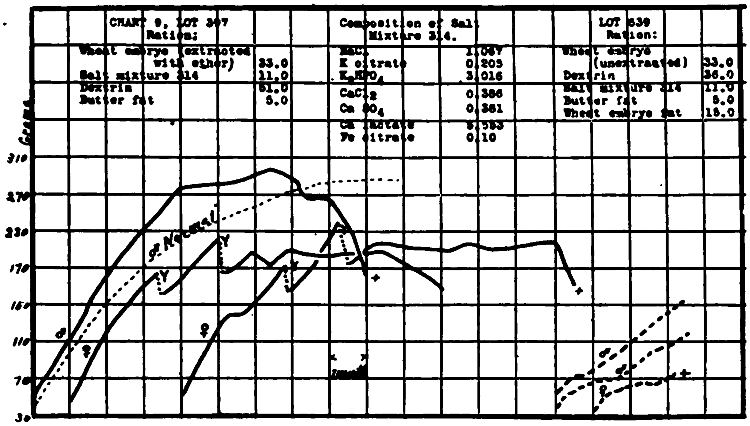


Chart 9. Lot 397 should be compared with Lot 639 whose rations were identical except that the wheat embryo in the latter was not extracted with ether and 15 per cent of the ether extract of wheat embryo was added instead of an equivalent amount of dextrin. Without the wheat embryo fats, growth progressed at the maximum possible rate. With these fats present growth was depressed to half the normal rate or less. The wheat embryo fats are distinctly toxic to animals, but this property will not be evident when fed with a highly satisfactory diet, except possibly through the accumulative effect over a long period.

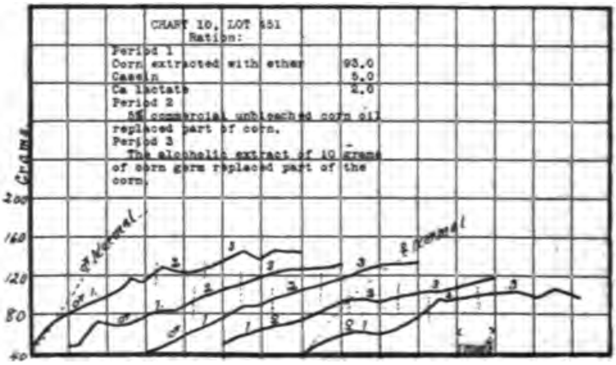


Chart 10. Lot 451, shows the property of ether extracted ground maize kernel, of promoting growth at a slow rate over a prolonged period. This, the ether extracted oil of corn, will not do. It should be noted that the inclusion of corn oil in period 2 or of the alcoholic extract of corn germ in period 3 did not produce any change in the direction of the curve of growth. The fat soluble A is present in the corn kernel but in amount below the optimum for growth, and the substance is not extracted by ether.

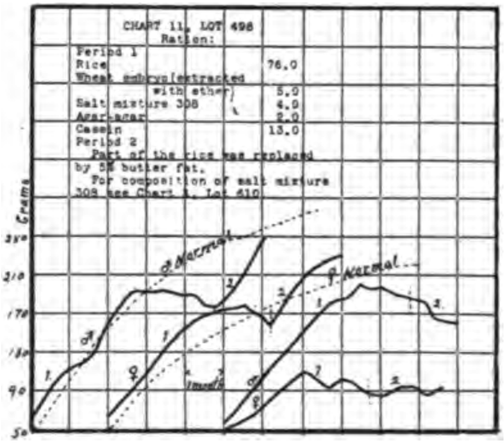


Chart 11. Lot 498. In this ration the only source of the fat soluble A was 5 per cent of completely ether extracted wheat embryo contained in the ration (period 1). During three months growth was normal but early failure ensued. That the shortage of the fat soluble A was the only factor responsible is shown by the prompt response of the two rats with growth when in period 2, 5 per cent of butter fat replaced 5 per cent of rice. There is no doubt but that this dietary factor is present in plant tissues but it seems unlikely that any isolated plant oil will contain it.

THE EFFECT OF CERTAIN PROCEDURES UPON THE COURSE OF FATIGUE IN FROG'S MUSCLE

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This research was suggested by the experiments of Lee and Salant (1), Hough and Ham (2), and Lee and Levine (3), in which attempts have been made to determine the effects of alcohol, of Ringer's fluid and of distilled water upon the course of fatigue in the excised muscle of the frog. In the experiments, as carried out by these observers, the thigh of one leg was ligated, the gastrocnemius of that leg removed and a fatigue tracing immediately made. Immediately following the application of the ligature about 2.5 cc. of water, Ringer's fluid or 10 per cent ethyl alcohol were injected subcutaneously and after allowing twenty to seventy-five minutes to elapse a tracing was made from the gastrocnemius of the other, or non-ligated leg.

In the experiments, as carried out by us, the procedure was the same as above except that in all cases the second tracing was taken from thirty to thirty-five minutes after the first, i.e., thirty to thirty-five minutes after the application of the ligature. In the cases in which a subcutaneous injection of fluid was made we used 2 cc. of Ringer's fluid or 2 cc. of 10 per cent ethyl alcohol injected into either the dorsal or anterior lymph sac.

Before the experiments had progressed very far, we noticed that in nearly every case the second, or "non-ligated" muscle did more work whether there had been a subcutaneous injection of fluid or not.

The rate of stimulation used was about thirty break induction shocks per minute, the current being interrupted mechanically and the make shocks short circuited. In a few cases the muscles were tetanized and the amount of work done obtained in this way. In all of the experiments in which the fatigue was brought on by single contractions supramaximal shocks were used and the muscle lifted a weight of about [illegible] grams. In the cases in which the muscles were thrown into tetanus [illegible] were used. It might be mentioned that the

TABLE 1

Relative amounts of work done by the excised gastrocnemii of frogs as shown by the total area of tracings in square centimeters

NUMBER OF EXPERIMENT	RIGHT LEG LIGATED	LEFT LEG NON-LIGATED	+ = PER CENT INCREASE IN WORK BY MUSCLE OF NON-LIGATED LEG — = PER CENT INCREASE IN WORK BY MUSCLE OF LIGATED LEG
2	108	135.3	+ 25.2
3	103.4	146.6	+ 41.7
4	109.2	114.4	+ 4.7
5	44.0	47.5	+ 7.9
6	51.1	63.0	+ 23.2
9	118.6	152.6	+ 28.6
13	100.0	154.6	+ 54.6
18	217.4	242.5	+ 11.5
39	109.7	134.4	+ 22.5
44	174.6	196.0	+ 12.2
45	96.2	110.1	+ 14.4
46	141.7	200.1	+ 41.2
66	125.7	136.0	+ 8.1
67	168.2	192.9	+ 14.6
72	64.0	105.2	+ 64.3
74	117.5	114.8	— 2.3
76	143.0	162.1	+ 10.2
78	156.5	174.0	+ 11.1
80	107.3	166.5	+ 56.6
94	98.2	107.0	+ 8.9
96	141.0	159.2	+ 12.9
98	138.0	142.8	+ 3.5
102	139.0	146.3	+ 5.25
103	102.7	146.6	+ 42.7
109	174.2	185.0	+ 6.2
113	248.3	257.7	+ 5.7
115	139.8	179.4	+ 28.3
119	84.0	134.0	+ 59.5
136	88.7	98.5	+ 11.1
137	58.4	53.5	— 9.1
138	64.3	144.3	+124.5
140	56.3	125.4	+122.7
141	87.9	115.6	+ 29.2
142	67.0	78.5	+ 17.1
143	75.4	98.0	+ 29.9
145	73.1	74.9	+ 2.4
146	35.0	72.5	+107.1
148	100.0	136.8	+ 36.8
149	120.0	129.6	+ 8.0
150	54.1	97.2	+ 79.6
151	70.1	71.5	+ 1.9
152	125.0	145.2	+ 16.1
153	94.4	141.0	+ 49.3
154	120.0	138.6	+ 15.5

TABLE 2

Relative amounts of work done by excised gastrocnemii of frogs as shown by the total area of tracings in square centimeters

NUMBER OF EXPERIMENT	LEFT LEG LIGATED	RIGHT LEG NON-LIGATED	+ = PER CENT INCREASE IN WORK BY MUSCLE OF NON-LIGATED LEG − = PER CENT INCREASE IN WORK BY MUSCLE OF LIGATED LEG
51	192.5	169.7	−13.4
52	111.7	88.0	−26.9
53	111.0	184.0	+65.7
54	166.9	173.8	+ 4.1
55	195.3	181.0	− 7.8
56	160.0	213.5	+33.4
57	166.2	215.4	+29.6
58	105.4	147.3	+39.8
59	176.3	169.8	− 3.8
60	162.4	179.9	+10.7
61	146.3	148.5	+ 1.5
63	147.6	151.2	+ 2.4
64	86.0	131.7	+53.1
65	190.3	163.7	−16.2
73	120.4	127.4	+ 5.8
75	84.0	111.0	+32.1
79	108.9	137.0	+25.7
81	205.3	200.8	− 2.2
86	88.5	120.8	+36.4
91	113.0	143.2	+26.7
95	87.8	150.2	+71.0
99	71.2	108.6	+52.5
107	180.6	199.5	+10.4
110	122.7	178.2	+45.2
114	214.9	225.6	+ 4.9
116	122.0	167.2	+37.0
117	57.5	61.3	+ 6.6
118	158.5	193.1	+21.8
123	97.3	87.0	−11.8
124	77.0	92.0	+19.4
125	66.4	115.0	+73.1
126	143.5	158.4	+10.4
128	34.0	44.0	+29.4
129	93.0	98.0	+ 5.4
130	77.5	104.0	+24.2
132	116.0	123.5	+64.6
133	98.9	136.2	+37.7
134	31.6	75.0	+137.3
135	72.2	92.0	+27.4
207	64.0	84.0	+31.2
208	38.3	43.7	+14.0
209	50.0	70.4	+40.8

TABLE 3

Relative amounts of work done by the normal gastrocnemius and by the gastrocnemius the other leg thirty to thirty-five minutes after injecting 2 cc., of 10 per cent alcohol into dorsal lymph sac

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	ALCOHOLIZED LEG LEFT LEG	+ = PER CENT INCREASE IN WORK BY ALCOHOLIZED MUSCLE — = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
8	49.6	109.7	+121.2
11	143.0	176.4	+ 23.2
15	129.4	139.0	+ 8.5
20	351.4	317.0	− 10.8
21	188.7	224.0	+ 18.7
23	211.5	219.0	+ 3.5
26	231.5	267.1	+ 15.3
32	227.4	264.5	+ 16.3
34	302.2	220.4	+ 37.1
35	159.0	214.2	+ 34.7
36	169.5	170.5	+ 0.6
48	170.5	167.5	− 1.7
69	187.6	181.7	− 3.2
82	234.4	239.4	+ 2.1
83	139.8	154.8	+ 10.6
88	90.5	117.6	+ 29.9
92	108.8	114.2	+ 4.6
100	148.0	153.5	+ 3.7
104	122.3	90.8	− 34.6
155	161.2	155.8	− 3.4
156	110.0	198.4	+ 80.3
157	172.6	154.1	− 12.0
158	185.2	208.4	+ 12.5
159	168.3	193.9	+ 15.2
160	155.4	200.8	+ 29.2
161	129.3	134.5	+ 4.0
162	115.6	150.6	+ 30.2
163	167.6	218.3	+ 30.2
164	160.7	151.3	− 6.2
165	118.3	125.4	+ 6.0
167	109.7	134.4	+ 22.4
168	161.3	180.3	+ 11.1
169	117.5	119.0	+ 1.2
170	57.3	56.6	− 1.2
171	205.9	213.1	+ 3.4
172	172.6	197.3	+ 14.2
173	188.2	186.2	− 1.0
174	160.6	192.6	+ 19.9

TABLE 3—Continued

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	ALCOHOLIZED LEG LEFT LEG	+ = PER CENT INCREASE IN WORK BY ALCOHOLIZED MUSCLE − = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
175	191.7	192.2	− 0.5
176	184.7	177.0	− 4.3
177	138.0	160.7	+ 16.4
178	122.7	124.4	+ 1.3
179	115.0	128.0	+ 11.3
180	123.7	147.7	− 11.0
12	74.5	185.1	+148.4
29	158.0	216.3	+ 36.8
33	118.3	168.5	+ 32.4
38	158.0	172.0	+ 8.8

TABLE 4

Relative amounts of work done by the normal gastrocnemius and by the gastrocnemius of the other leg thirty to thirty-five minutes after injecting 2 cc. Ringer's fluid into the dorsal lymph sac

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	AFTER RINGER'S LEFT LEG	+ = PER CENT INCREASE IN WORK DONE BY THE MUSCLE AFTER RINGER'S FLUID − = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
7	112.0	205.8	+ 83.7
10	68.7	128.0	+ 87.7
14	76.5	219.0	+186.2
17	210.9	190.4	− 10.7
22	298.3	249.8	− 19.4
24	217.0	220.3	+ 1.5
25	197.0	192.4	− 2.3
27	236.2	216.0	− 9.3
31	179.7	143.0	− 25.6
37	173.3	217.9	+ 25.7
40	162.0	150.3	− 7.7
41	147.3	152.4	+ 3.4
42	128.5	106.5	− 20.6
43	114.5	103.0	− 11.1
47	153.7	166.0	+ 8.0
50	152.7	118.4	− 28.1
70	95.0	122.9	+ 29.3
85	119.2	143.7	+ 20.6
89	107.5	123.3	+ 14.7

TABLE 4—Continued

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	AFTER RINGER'S LEFT LEG	+ = PER CENT INCREASE IN WORK DONE BY THE MUSCLE AFTER RINGER'S FLUID — = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
93	123.4	111.0	− 11.1
97	110.2	150.8	+ 50.4
101	198.4	153.7	− 22.5
105	108.3	125.0	+ 15.4
182	119.4	140.4	+ 17.6
183	155.3	151.5	− 2.5
185	148.0	192.9	+ 30.3
186	127.8	147.0	+ 15.0
187	82.4	98.5	+ 19.5
189	136.7	123.0	− 11.1
190	113.0	127.5	+ 12.8
191	122.5	130.1	+ 6.1
192	128.9	126.0	− 2.4
193	126.4	131.5	+ 4.0
194	126.7	151.1	19.2
195	172.3	170.1	− 1.3
196	133.0	136.0	+ 2.2
197	144.7	150.0	+ 3.6
198	145.4	167.7	+ 15.3
199	157.5	166.4	+ 5.6
200	201.4	206.5	+ 2.5
201	172.0	171.1	− 0.5
202	236.7	240.4	+ 1.5
203	121.0	171.6	+ 41.8
204	170.5	208.1	+ 22.0
205	191.5	168.3	− 13.7
206	140.5	189.1	+ 34.5

two methods gave practically the same results so far as the amount of work done was concerned. The total amount of work done was determined by measuring the total area of the tracings with the planimeter as suggested by Hough and Ham (2).

The above tables give a summary of the experiments done.

RESULTS

In table 1, we give the per cent increase or decrease of work done by the muscle of the left leg which was the second, or non-ligated leg. In this table in which there are 44 experiments the average per cent of increase of the non-ligated muscle is 25.7[illegible]. In this table, it will be noticed that in only two cases did the muscle of the second or non-ligated leg fall below that of the first or ligated leg.

In table 2 the right leg is the non-ligated leg. In this table, in which there are 42 experiments, the per cent of increase of the right muscle, which is the muscle of the non-ligated leg, is 25.22. In this table in only seven cases did the muscle of the second leg fall below that of the first.

In table 3, in which 48 experiments were carried out, the per cent of increase by the non-ligated, or "alcoholized" muscle is 16.84. In this table there are nine cases in which the amount of work done by the alcoholized muscle is less than that by the normal muscle. In all but four cases we used the left leg for the alcoholized leg.

In table 4, in which 46 experiments were carried out, the per cent of increase by the muscle of the second leg following the absorption of Ringer's fluid is 12.61. In this case, it will be noticed that there are sixteen experiments in which the second muscle did less work than the muscle of the normal leg.

We found in about two-thirds of the cases of the alcoholized muscle that the initial contraction is slightly less in height than that of the normal muscle; however, the same is found to be true in the second muscle after the injection of Ringer's fluid or even after the other leg has been ligated for thirty minutes and no fluid injected. The phenomenon of introductory contractions was present in only a few cases.

The "stair-case" is more gradual and the maximum is reached later in the muscle of the non-ligated leg than in the normal muscle whether there has been a subcutaneous injection of fluid or not. The height of contractions and the number of individual contractions is, in the majority of cases, greater in the muscle of the second, or non-ligated leg. The oncoming of fatigue and the appearance of exhaustion is delayed in the majority of the cases in the muscle of the non-ligated leg. In other words, the results heretofore claimed for alcohol and Ringer's fluid are found to be present in the case of the muscle from the second leg whether there has been a subcutaneous injection of fluid or not. All of these results are as marked in the cases of comparison as in the cases following alcohol or Ringer's fluid; the increase in the amount of work done by the second muscle is greater when there is no injection of alcohol or Ringer's fluid.

It was noticed, as reported by Lee and Salant, that the increase in amount of work done by the second muscle is greater in the case of winter frogs. This is true in the cases of comparison as well as following alcohol or Ringer's fluid. We did not find contracture more pronounced in the normal muscle. The early appearance of contracture we found depended upon the strength of the stimulus used. In those cases in which we tried to use a supra-second-maximal stimulus, we found that the muscle went into an early and marked contracture. In the cases in which the stimulus was not above the second maximum, there was no early contracture. In a few cases there was a delayed contracture, even with the rate of stimulation of 30 per minute.

CONCLUSIONS

1. The ligation of one leg increases the working power of the excised muscle of the other leg after a wait of thirty minutes.
2. That, while the alcoholized muscle or the muscle of the leg following the absorption of Ringer's fluid does more work than the normal muscle, it does this work, not on account of the effect of the alcohol or Ringer's fluid, but as the result of the ligation of the other leg.
3. Both alcohol and Ringer's fluid have reduced the working power of the muscle.
4. The method described cannot be used to determine the effect of drugs on the working power of an excised muscle.
5. It is suggested that the ligation of one leg may bring about an increase in the working power of the muscle of the other leg by reflexly causing an increase in blood pressure and thus improving the circulation through the muscle of the non-ligated leg during the thirty minutes wait. It may be that the effect noticed is partly due to secretion of adrenalin following the operative procedure. These are simply suggested as possible explanations of the results noticed.

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(1) Lee and Salant: This Journal, viii, 1902, 61.
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THE BLOOD PRESSURE IN EXPERIMENTAL PNEUMONIA

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In 1914 Newburgh and Minot (1) made numerous observations of the blood pressures in pneumonia in the wards of the Massachusetts General Hospital, to which were added thirty cases from the Johns Hopkins Hospital. It was found that the average of readings taken morning and afternoon during the course of the disease was from 110 to 120 mm. Hg. The normal for human beings of this age is 120 to 130 mm. In the fifteen cases observed in the Massachusetts General Hospital the lowest pressure was 90 mm. There were three cases at 100 mm.

These clinical studies were made to determine the prognostic value of the blood pressure in pneumonia. It was felt at the time that since the pressures did not fall during the course of the disease, nothing would be gained as an indication for treatment by recording the pressure two or three hours before death. It would then be too late for therapeutic measures. The teaching at that time was that persons who were to die of pneumonia would have a low or falling pressure days before the fatal issue.

In the course of our studies in experimental pneumonia we frequently observed that the carotid artery seemed to contain less blood than usual, a few hours before death. It was therefore of interest to measure the blood pressure in experimental pneumonia (2), and especially to follow it to the end.

We present in table 1 the results of our observations. The measurements above the horizontal line are from the curves recorded in the work upon "The State of The Vasomotor Apparatus in Pneumonia," (3) those below the horizontal line were made for this present paper, especially to determine the pressure just preceding death.

It will be observed in table 1 that while the pressures are somewhat lower than normal, there is but one case in which the fall was such as to endanger life; and this dog, No. 12, was measured literally at the

TABLE 1

The blood pressure in experimental pneumonia

ANIMAL	NUMBER	TEMPERATURE	BLOOD PRESSURE		TIME BEFORE DEATH	REMARKS
			Diastolic	Systolic		
Cat......	2	38°C.		105	6 hrs.	
Cat......	4	41		85	3 hrs.	
Cat......	11	40		210	5 min.	Death due to ether.
Cat......	13	34		150	2 hrs.	
Dog......	8	40		135	3 hrs.	Experiment was ended by killing animal.
Dog.....	12	34		30	2 min.	At point of death when pressure was measured.
Dog.....	13	40		100	2 hrs.	
Rabbit...	51	40		82	2 hrs.	
Dog.....	109	36	85		25 min.	
Dog.....	110	36	85	105	15 min.	
Dog.....	111	39.5	75	100	40 min.	
Dog.....	112	40.5	50	85	1 hr.	
Dog.....	113	39.5	45	70	25 min.	
Dog.....	114	37	60	85	45 min.	
Dog.....	115	38.3	45	85	70 min.	
Dog.....	116	39.3	60	90	5 hrs.	Two measurements from same dog.
Dog.....	116	39	48	80	2 hrs.	

TABLE 2

The blood pressure in pneumococcus septicaemia

ANIMAL	NUMBER	TEMPERATURE	SYSTOLIC BLOOD PRESSURE	HOURS BEFORE DEATH
Rabbit..........	2	41	100	3½
Rabbit..........	3	40	102	3
Rabbit..........	6	40	80	2
Rabbit..........	9	40	85	1
Rabbit..........	10	40	75	2
Rabbit..........	13	41	100	1½

point of death, which took place not more than two minutes after the record. On the other hand, in the experiment of April 7, the dog had a pressure of 105 mm. fifteen minutes before his death.

It will be interesting to compare the records of table 1 with those of table 2 in which the pneumococcus septicaemia was produced in six rabbits by injecting the organism into the vein. This procedure is followed by the same bacteraemia observed in fatal cases of pneumonia but without any inflammation of the lungs. In these cases the pressure is very satisfactory even two hours before death.

CONCLUSION

The blood pressure in experimental pneumonia is frequently somewhat less than normal, but, in our experience, has never been so low as to endanger life.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

I. THE OCCURRENCE AND MEASUREMENT OF A SUDDEN CHANGE IN THE RATE OF GROWTH OF AVIAN OVA

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The physiology of reproduction has always been of interest and importance to the practical breeder. It is only recently however that this branch of physiology has shown itself to be so intimately connected with important and varied functions of the organism as to command much attention from physiologists generally. More recently still it is becoming clear that further advance in unravelling the complicated processes of heredity is, in part at least, dependent upon a closer knowledge of the *exact conditions* under which the germinal elements form, grow, function, mature, unite, and take the first steps of embryonic development.

Our choice of material has been determined by two circumstances which combine to make it seem specially desirable at this time to amplify at several points our present information on the physiology of reproduction in birds. In the first place, very extensive studies involving perhaps the greatest amount of control of hereditary processes yet attained have been carried out upon these forms[1] by Whitman, and later by myself (1, 2, 3); furthermore, new and exact data on the storage metabolism of the ova of pigeons—the identical material used by Whitman—have now demonstrated (3, and unpublished data) not only a correlation of this metabolism with the sex of the offspring, but a fact that necessarily follows, namely, that this high or low degree of storage in the ova—and thus the sex—cannot be wholly independent of the metabolism of the organism. In the second place, the ovary, and many of the individual ova, of the bird are large enough to permit of individual study, and of methods of study hardly available elsewhere among animals.

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[1] Only a fraction of the data have as yet been published.

It happens therefore that avian reproduction supplies a gateway through which some of the results and methods of modern physiology may be brought to bear upon a fundamental problem of heredity and of sex.

Primary oöcytes are known to be already formed in a female chick one week old, and in several species from widely separated groups of birds it is known that the oögonia are formed at a correspondingly early period. It is well known too that a "growth period" marked by the deposition of yolk begins at once. In general this growth continues until the maturation of the egg, and its extrusion from the ovary.

It is also generally understood[2] that the rate of growth is not uniform, or continuous, and may be divided into a number of periods. In a previous study (7) I found some evidence that the growth period in the fowl is not a steady, unbroken one—not even among the larger continuously growing ova of a functioning ovary; but at a certain point the rate quite suddenly changes to a rate apparently eight to twenty times faster than before; and further, that the change in rate normally occurs five to eight days before ovulation, and when the ova have reached a diameter of about 6 mm.

It seemed desirable further to study and to test these indications since if such a radical change in growth really occurs it is probably a fact of importance in the origin of the germ cells; furthermore, the changes seemed to occur at a stage when the egg is large enough (6 mm.) to be available not only for the usual cytological study, but for macroscopical observation and physiological experiment as well.

The earlier evidence indicative of such change in the growth-rate was of two kinds: First, ova of the fowl which were less than 6 mm. in diameter failed to take up Sudan III from the blood stream;[3] while growing ova of more than 6 mm. in diameter readily took up the dye. The second kind of evidence was obtained by a comparison of the thickness of striations found in one small ovum (7 mm.) with other striations generally present in larger ova. We quote as follows from our former paper (l.c., p. 460):

[2] See D'Hollander (4), Sonnenbrodt, (5), Bartelmez (6) and others.

[3] I.e., not in amounts macroscopically observable. I have elsewhere (7) described in detail the use of Sudan in the measurement of the rate of growth of ova.

> Here (in the 7 mm. ovum) the noteworthy facts are, that a striation exists, and that the lamellae are not thicker than 0.25 mm. Whether these lamellae are made up of still smaller strata which really represent days of growth I am quite unable to say. I doubt somewhat that the radius of these small eggs is increased by as much as 0.25 mm. in twenty-four hours; anyway these strata offer some evidence—in the light of what we know of succeeding yolk strata—that these small eggs do not grow *faster* than 0.25 mm. per day (the larger eggs grow at the rate of 2 mm. per day).

From indications then at hand it seemed pertinent (p. 460) to ask

> What is the meaning of the extraordinary difference in growth-rate of eggs under, and over, 6 mm. in diameter? What old mechanism is inhibited, or what new one brought into action, that will account for this procession of cells—each with *months* of slow and constant growth behind it—coming to a point from which it jumps in a *day* from its accustomed rate of increase, to a rate that is probably from eight to twenty times higher? Do the follicular cells now become more permeable than formerly to the ingredients of yolk? Is the increased vascularity of the follicular envelopes, that certainly occurs at this time, a cause or a result of the new activity?

In considering methods of obtaining positive and crucial evidence on the question of the existence and amount of such difference in growth-rate it was seen that if the eggs of more than 6 mm. diameter grow twenty times as fast as the eggs of smaller diameter, then the number of eggs above and below 6 mm. in a functioning ovary should, at any time, bear a 1 : 20 ratio to each other. That is, there should be twenty times as many ova under 6 mm. *included in a given unit of radius*—say 1 mm. or 3 mm.—as there are ova of more than 6 mm. within the same unit of radius. Or, concretely, there should be twenty times as many eggs between 3 and 6 mm. in diameter in a ovary as there are eggs between 6 and 9 mm. in diameter in the same ovary. It follows therefore that one can thus count and classify the ova in a number of appropriate ovaries, and then *calculate* the difference in rate of growth in the two groups of ova.

In actual practice all the ova above 6 mm. diameter have been counted and this total number compared with the number of eggs of 3 to 6 mm. diameter in the same ovary. Considering the final size of the eggs to be 30 mm., the rapid growth period involves the addition of 24 mm. From these data the relative rate of growth is obtained from two simple proportions.[4]

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[4] E.g., if (as in ovary No. 1) there are 5 of the larger ova (= 24 mm. growth) and 19 smaller ones (= 3 mm. growth), we find, 3 : 19 : : 24 : x; x = 152; then 5 : 152 : : 1 : x; here x = 30.4 the number sought.

For this study were used only fresh ovaries known to be actually discharging ova—as shown by egg laying record, by eggs in oviducts, or by freshly opened follicles. Most of the material was obtained from a butcher near by who permitted the removal of the ovaries from the birds as soon as they were killed. Eight of those studied and tabulated here were our own birds, whose egg-laying history was known. During two summers nearly two hundred ovaries were examined; complete and exact measurements were however made on only forty of these.

The result as determined upon this material by this method of calculation is subject to error from the following sources: (1) A functional ovary from an old bird may be nearly approaching the extrusion of the last ovum this bird can ovulate; in this case the calculated rate of growth may be much too low (see Nos. 4–23–27–30 in table 1). (2) The largest ovum may have escaped from the ovary but a few hours before death. These few hours might bring no change in the number of ova measuring 3 to 6 mm., but would change the number above 6 mm. by one-sixth of the total. This is probably the true history of nearly half of the ovaries here tabulated (since only ovaries from laying hens are considered), and tends to make the calculated result too high. It has therefore been thought wise to base the calculation upon the actual number found, and then upon this actual number plus one (see column 6, table 1). The actual rate more probably lies between these two figures. (3) The occurrence of follicular atresia, or the resorption of ova, may modify the result in either direction; particularly if the larger resorbed ova become confused with degenerating capsules from which eggs have been liberated. Where such conditions were confusingly in evidence we have rejected the material. (4) In a few cases it has been found difficult to know whether the largest of the slow-growing eggs has yet begun to deposit yellow yolk—that is, whether it has already started the new rate of growth; and in two cases (see Nos. 17 and 21, table 1) there were two and three respectively of such ova of doubtful classification. Error in making this classification may modify the result in either direction. (5) The final diameter of the yolk of the fowl is not uniformly 30 mm.; probably it is oftener more than less. If so, the actual sudden increase in rate of growth of ova when they reach 6 mm. diameter is somewhat greater than is indicated by our figures.

A diameter of 6.0 mm. was not always and arbitrarily taken as the dividing point, but in each ovary this point was determined by the diameter of the largest ovum with no yellow yolk evident at its surface. If this proved to be 6.5 mm. the number of ova between this and 3.5 mm. was determined and used in the classification.

The data of tables 1, 2 and 3 seem to establish beyond question the fact of a sudden increase in the growth-rate of ova of the fowl when these attain a diameter of about 6 mm. It is further shown that the extent of this increase is somewhat greater than our earlier evidence

TABLE 1

I		II	III	IV	V	VI
June	14	1	5	19	30.4	25.3
	14	2	8	17	17.0	15.1
	14	3	7	19	21.7	19.0
	14	4	4	3	6.0	4.8
	14	5	6	21	28.0	24.0
	14	6	6	27	36.0	30.9
July	3	7	5	20	32.0	26.7
August	3	8	5	28	44.8	37.3
	3	9	6	27	36.0	30.9
	3	10	7	19	21.7	19.0
	17	11	5	24	38.4	32.0
	17	12	6	20	26.7	22.9
	22	13	6	20	26.7	22.9
	22	14	4	19	38.0	30.4
	22	15	5	32	51.1	42.6
	24	16	4	12	24.0	19.2
	24	17	4 (2)	8	20.0	18.0
					16.0	14.5
					10.7	9.9
	24	18	4	17	34.0	27.2
	24	19	5	12	19.2	16.0
	28	20	6	14	18.7	16.0
	28	21	5 (3)	21	38.4	32.0
					33.6	28.0
					21.0	18.7
	31	22	6	22	29.3	25.1
	31	23	4	10	20.0	16.0
September	7	24	5	17	27.2	22.7
	7	25	4	20	40.0	32.0
	7	26	7	16	18.3	16.0
	7	27	6	8	10.7	9.1
	14	28	7	14	16.0	14.0
	14	29	5	15	24.0	20.0
	21	30	5	9	14.4	12.0
	21	31	4	21	42.0	33.6
		Av. =5.4		18.9	28.0	23.6

The several columns bear the following data: I = date of examination of ovary; II = serial number of ovary; III = the number of ova more than 6.0 mm. diameter in ovary; IV = number of ova between 3.0 and 6.0 (approximately) mm.; V = calculated difference in rate of growth of the two groups of ova; VI = this difference of rate when (1) is added to the number of larger ova actually found (see text). Average does not include Nos. 4–17–21–27. The dates refer to 1911 and 1912.

had suggested; the present data indicating the rate to be between 23.6 and 28 times more rapid in the larger ova. The average of these two numbers, i.e., 25.8, probably very nearly measures the remarkable extent of this sudden jump in growth power.

That the whole of this saltation in rate of growth may be completed in as short a space of time as a day is shown by an examination of the data presented in tables 2 and 3 taken in connection with the following considerations: (1) The commonly observed fact that one of the largest ova may be ovulated daily. (2) In such cases an egg of the 6 mm. group must daily join the restricted group of the larger ova. Now, the size differences of the very restricted number of eggs immediately over 6 mm. in diameter indicate (tables 2 and 3) that the full extent of the new growth power is often attained in a single day.[5] Convincing evidence of another kind on this point resulted from a direct measurement (7, p. 459) with our Sudan III method of studying the rate of growth of the more central and peripheral yolk of the hen's ovum. The indicated rates of growth were 1.53 mm. for the more central[6] portions, and 1.67 mm. for the peripheral portions of yolk per twenty-four hours. The difference here is within the limits of error of measurement. The first yolk that is added to an ovum already 6.0 mm. in diameter is therefore added at about twenty-five times the previous rate.

It is of some interest to note the part that seasons play in our data on the indicated increase in rate of growth. This rate seems higher in the early summer: 27.4 = average of first ten ovaries; and lower in late summer: 21.6 = average of last ten ovaries. It is doubtful however whether importance attaches to these figures.

The change in the rate of deposition of yolk involves the follicular membrane—which everywhere covers the surface of the ovum—in a way that is interesting to note. The radius of the ovum is increased in the period of rapid growth by nearly 2.0 mm. in twenty-four hours. This requires, during each thirty minutes or less, the passage through (or between) each of these follicular cells of a quantity of yolk material equal to the volume of the cell itself. The material thus transported

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[5] Ovaries No. 2–6–8–17–26 on the other hand show two of these ova of nearly the same size, and No. 21 contained 3 such ova of nearly equal size. But these constitute only one-fifth of the total and we know that hens sometimes lay two eggs within less than twenty-four hours, and that they also sometimes lay double-yolked eggs. Follicular atresia too may eliminate some.

[6] I.e., parts immediately *external* to the 6 mm. central sphere of white yolk.

TABLE 2

Showing detailed measurements, in millimeters, on eight ovaries (early summer) tabulated in table 1. The line separates eggs growing at vastly different rates

NO. 1	NO. 2	NO. 3	NO. 4
32 × 34 30 × 33 25 × 30 = 5 13 × 15 9 × 10 ———— 6 × 6 5 × 6 = 3† 4½ × 5 4 × 5 = 19 4 × 4 = 4 3½ × 4 = 2 3 × 4 3 × 3 = 6	28 × 30 25 × 30 24 × 27 = 8 20 × 22* 13 × 17 10 × 18 8 × 8 6½ × 7 ———— 6 × 6 = 3 5½ × 6 5 × 5 = 3 = 17 4½ × 5 = 2 3 × 4 = 2 3 × 3½ = 2 3 × 3 = 4	28 × 33 26 × 28 25 × 27 = 7 23 × 24 21 × 25 16 × 18 6 × 6½ ———— 5½ × 6 = 2 5 × 5 4½ × 5 = 2 4 × 4 = 3 = 19 3½ × 4 = 4 3 × 3½ = 4 3 × 3 = 3	32 × 35 24 × 27 22 × 23 = 4 16 × 18 ———— 5 × 5 3½ × 4 = 3 3 × 3
NO. 5	**NO. 6**	**NO. 7**	**NO. 8**
25 × 29 23 × 26 19 × 20 = 6 18 × 19* 15 × 18 7 × 7½ ———— 6 × 6 = 2 5½ × 6 5½ × 5½ 5 × 5½ = 2 5 × 5 = 3 = 21 4½ × 5 4½ × 4½ = 2 4 × 4 = 3 3½ × 4 = 2 3 × 3½ = 2 3 × 3 = 2	23 × 27 22 × 28 15 × 18 = 6 13 × 14 8 × 8½ 6½ × 8 ———— 6 × 6 5 × 6 = 2 5 × 5 = 3 4½ × 5 = 2 4½ × 4½ = 27 4 × 4 = 4 3½ × 4½ 3½ × 4 = 3 3 × 3½ = 4 3 × 3 = 4	28 × 33 26 × 29 20 × 23 = 5 14 × 17 8 × 11 ———— 6 × 6 5½ × 6 5 × 6 = 3 5 × 5 = 2 4½ × 5 = 3 4½ × 4½ = 20 4 × 4½ = 3 4 × 4 3½ × 4 = 2 3½ × 3½ 3 × 3 = 2	28 × 30 21 × 25 12 × 14 = 5 7 × 8 6 × 7 ———— 6 × 6 5½ × 6 5 × 5 = 4 4½ × 5 = 4 4½ × 4½ = 4 = 28 4 × 4 = 3 3½ × 4 = 3 3 × 3½ = 3 3 × 3 = 4

* Bag-like.

† The numbers in this column indicate the number of eggs corresponding to a given measurement.

TABLE 3

Showing detailed measurements on eight ovaries (autumn) tabulated in table 1. The line indicates the point of change in growth-rate

NO. 24	NO. 25	NO. 26	NO. 27
29·× 35	27 × 31	30 × 33	27 × 32
27 × 30	22 × 25	25 × 31	24 × 29
25 × 27 ≐ 5	15 × 17 = 4	23 × 29	22 × 25 = 6
20 × 24	11 × 12½	21 × 24 = 7	18 × 21[*]
16 × 18	———	15 × 20	18 × 21
———	6 × 7	11½ × 14	9 × 10½
6½ × 7½	6 × 6½	10½ × 12½	———
6 × 7	5½ × 6½	———	5½ × 6½
+6 × 6½	5½ × 6 = 2	6 × 6½	5 × 6
6 × 6½·	5¼ × 6 = 2	5½ × 6¼	5 × 5½
5½ × 6	5¼ × 5½	4½ × 5½	5 × 5¼ = 8
5 × 5½ = 2 = 17	5 × 5½ = 20	4½ × 5½	4½ × 5
4½ × 5 = 2[†]	4½ × 5½	4½ × 5 = 2 = 16	3¼ × 3¼
4 × 5 = 2	4½ × 5	4 × 4½ = 2	3 × 3 = 2
3¾ × 5	4½ × 4¾	3½ × 4 = 2	
4 × 4 = 2	4 × 4½	3½ × 3½ = 2	
3¾ × 4	3½ × 4 = 3	3 × 3½ = 2	
	3 × 3¾ = 3		
	3 × 3½		

NO. 28	NO. 29	NO. 30	NO. 31
28 × 32	30 × 33	30 × 33	29 × 33
27 × 29	22 × 24	19 × 22	24 × 28
22 × 27	17 × 19 = 5	16 × 18 = 5	16 × 19 = 4
20 × 22 = 7	12 × 13	11 × 13	7½ × 8
15 × 18	7½ × 7½	7 × 8½	———
12 × 13½	———	———	5½ × 5½
8½ × 9½	5½ × 5¾	6 × 6½	5 × 5½
———	5 × 5¾	5 × 5	4¾ × 5
5½ × 6½	5 × 5½	3½ × 4	4½ × 5 = 2
5 × 6	4½ × 5 = 15	3 × 4[‡] = 9	4½ × 4¾ = 21
4 × 5½	4½ × 4½	3 × 3¾	4 × 4 = 3
4½ × 5 = 14	4 × 4½	3½ × 3½ = 2	3½ × 4 = 3
4 × 4½	4 × 4 = 3	3 × 3¼ = 2	3¼ × 3½ = 3
3½ × 3½ = 3	3¾ × 4		3¼ × 3¼
3 × 3½ = 2	3½ × 3½ = 3		3 × 3¼ = 3
3 × 3 = 4	3¼ × 3½		3 × 3 = 2

[*] Not absolutely certain this egg belonged in this ovary.

[†] The numbers in this column indicate the number of eggs corresponding to a given measurement.

[‡] Degenerating.

is water-poor, and rich in fat. At this same time moreover many new follicular cells must be formed. Within as short a time as five days a 6 mm. ovum may have grown to 33 mm. The relative sizes of the membranes for ova of the two stages are as 1 to 30. If all of the follicular cells arise from follicular cells—as seems probable—each cell must divide every twelve hours (10 divisions in 120 hours). It is certain that young and growing cells are abundant in the follicular membrane during the period of the rapid growth of the ovum. The chemistry of this membrane in the various phases of its activity, and the chemistry of the two forms of yolk which correspond to the two rates of growth here described, are the subjects of investigations to be published immediately in this series of papers.

It remains to point out that a strictly comparable change in growth-rate occurs during the "growth period" of other avian ova—very clearly in the pigeons—and indeed in the ova of many animals. The *time* of the change is most variable for the ova of different classes and phyla; but the extent of the change is probably nowhere as pronounced as in the birds.

His (8, p. 20) distinguished by their macroscopic appearance four classes of ova (Follikel) in the hen's ovary. The present data, and the data of an earlier paper (7) show that there are only the two above-mentioned natural groups when these are considered from a dynamic standpoint. It was further noted by His (p. 5) that the "number of follicles, even in hens that laid every day, is strictly limited." He tabulated the contents of three ovaries, finding in them 5–3–5 eggs respectively of more than 9.0 mm. in diameter.

D'Hollander (4) and Sonnenbrodt (5) have carefully studied the development of the ovum in the fowl and divided its growth into more or less distinct periods; but chiefly on the basis of nuclear phenomena. Sonnenbrodt (p. 418) describes also the appearance of the hen's ovarial egg and notes that they are "whitish till of about 5 mm. diameter . . . . the smaller ones rather grayish . . . . ; over 5 mm. were very yellow." This observer further found "up to six follicles over 10 mm. diameter, but mostly three or four."

Bartelmez (6) describes four stages in the development of the ovarial egg (pigeon). His classification is based upon the phases of activity of the oöcyte as a whole, and from any point of view in which size and changes in growth are being considered, his treatment is certainly the best the subject has received.

Sonnenbrodt and Bartelmez report a slow growth of the oöcytes to about 0.09 mm. where they (the earliest, at least) halt. The next period, according to Bartelmez carries the egg to a diameter of 0.4 mm.; but between this and 5.0 mm. no change in rate of growth is suggested. From 5 mm. to ovulation he finds the rapid growth which I had previously shown to begin at about 6 mm. in the fowl.

Agassiz and Clark (9) long ago pointed out that there is a final growth period in the eggs of turtles which is more rapid than that which preceded it; this more rapid growth, lasting four years in all eggs; the first set of eggs beginning this rate in animals seven years old. In Lacerta, Braun and later Hoffman (10) showed that the formation of groups of ovarian follicles, with increased rate of growth, occurs only in the spring.

Many forms are known in which it has been practically certain that the final growth period is accomplished at an accelerated rate. A means of measuring this acceleration has however not hitherto been found. Moreover, the point at which it occurs has not been defined. Indeed, the term "final growth period" seems to have been rather generally applied to all the macroscopically visible ova of the ovary.

CONCLUSIONS

By suitable methods it has been possible to show that when the oöcyte of the fowl reaches a diameter of about 6 mm. it increases its previous rate of growth to a rate nearly 25.8 times higher.

The transition from the one rate to the other is made in a single day.

The transition sharply marks off the type of substance accumulated in the ovum. Under the slower rate this is white yolk; under the greatly increased rate the yellow yolk is produced.

The increased rate of deposition of yolk materials is necessarily accompanied by a pronounced alteration in the growth and activity of the membrane (follicular) which surrounds the ovum.

A comparable change in growth-rate occurs generally in other avian and sauropsidan eggs.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

II. ON THE CHEMICAL COMPOSITION OF WHITE AND YELLOW EGG YOLK OF THE FOWL AND PIGEON

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SOURCES OF INTEREST IN THESE DATA

The eggs of birds are known to contain yolk of two kinds. Because of their color the two kinds are called white and yellow yolk. The histological differences between the two forms are very marked and have long been known. The chemical differences are, however, known or indicated by only[1] a single partial analysis of white yolk reported by one of us (R. 1). The reason for the lack of analyses of the white yolk is doubtless to be found in the fact that the preparation of such samples is a most tedious process. Indeed, unless advantage is taken of the fact that the ova of the fowl consist wholly of white yolk until they begin their final period of rapid growth—described in Number I of these studies (2)—it is practically impossible to obtain such samples. When it is considered that the egg-nucleus, the germ, and the embryo are in direct contact with white yolk exclusively it becomes clear that we can know the chemical nature of the environment of the germs of birds only when the chemical composition of the white yolk is known. The analyses here reported demonstrate that the two forms of yolk are strikingly different substances, and that the white yolk much the more nearly approximates the composition of small holoblastic eggs, and of living undifferentiated tissue generally.

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[1] The impression (e.g., Hammersten, Physiological Chemistry) that Liebermann (Pfluger's Arch. vol. 43) made even a qualitative analysis of white yolk seems to be an error. The blastodisc (Keimscheibe) which he thus investigated is a quite different structure and material.

An examination of the available data on the percentage composition of the yellow yolk indicates that the moisture, neutral fat and protein figures warrant new determinations. And, if a comparison is to be made with the figures obtained for the white yolk it is particularly desirable that the analyses of the two substances be carried out by identical methods. The phosphorus compounds of the egg have been much studied, but our analyses include determinations of alcohol-soluble water-insoluble phosphorus, as well as figures for the total extractive bodies, and ash. In nearly all previous analyses it seems practically certain that a recently established fact of importance has been much disregarded; namely, the fact that water is continually passing from the albumen to the yolk from the time the ovum leaves the ovary till the time of preparation of the sample. Greenlee (3) has shown that this exchange occurs from the time an egg is laid until many months thereafter, and that the rate of such absorption of water by the yolk is much greater in the very fresh egg than in the stale one. Our own data show furthermore that in a "fresh egg"—that is, in any "laid" egg—the yolk has already been very appreciably diluted by water from the albumen. The ovarian yolk contains less moisture than the yolk of the fresh laid egg. In few of the previous analyses is the exact age of the egg considered. This means that the data from those analyses represent the percentage of water as being both higher and more variable than is true for samples of pure undiluted yolk.

Little is known of the variation in chemical composition of the yolk produced by different orders, species and races of birds. This is true though there are some available data on the yolk of various *breeds* of the common fowl, and though Langworthy (4) has made four useful analyses of yolks from other species of poultry. Before convincing evidence on this point can be had it seems necessary that eggs not only fresh but of *identical* age (in hours), be taken for comparison; that eggs from more than one individual of a breed be used; and finally that the age, and previous egg-laying history of each bird be known and considered. These last-named points are justified by the data from about nine hundred analyses of individual yolks from eggs of various species of pigeons and doves, as this has been carried out by one of us (Riddle, unpublished data). A comparison of the chemical composition of the egg yolk of birds belonging to two different sub-orders of birds is afforded by the present data on the fowl and the pigeon—the analyses for both kinds of eggs having been carried out by a uniform method.

A further reason for interest in a comparison of the composition of the two forms of yolk lies in the fact that the two forms have been shown (1 and 2) to be associated with two strikingly different growth rates; the two substances arise in the same cell under two quite unlike metabolic conditions. Our analyses show that the white yolk—which is stored but slowly in the cell—has a percentage composition approximately that of living growing tissues; and that the yellow yolk which is stored in the cell at a rate that is 25.8 times greater (see Number I of these studies) departs very widely in composition from all other substances or tissues.

METHODS OF PREPARATION AND ANALYSIS

The samples of yellow yolk from the laid eggs of common fowls and Jungle fowls were prepared as follows: The shell was divided along its longer diameter with a pair of small scissors. The two halves were used as 'boats' in which, alternately at first, the yolk was floated until freed from the greater part of the albumen. When a part of the albumen was thus removed the two chalazae were carefully cut away from their point of union with the vitelline membrane. The complete or final removal of albumen was accomplished by the use of elongated strips of hard filter paper which were repeatedly rolled under the yolk while still in its boat. In this way it is believed that all traces of albumen were removed from the yolk and from that half of the shell from which the yolk was finally released into the large weighing tube. The ovarian eggs were taken from birds as soon as these were dead. The outer theca was stripped from the ripest ovum; two samples (Y, Z) were then obtained by slitting the follicle at the hilum and forcing the last traces of yolk from the surrounding follicle. The third of these samples (M) was analyzed together with its inner follicular membrane as noted elsewhere.

The yellow yolk of the pigeon's eggs was obtained by steaming the just-laid eggs for eight minutes, cooling in water for four minutes, and then completely removing the albumen and vitelline membrane. These samples are quite clean, and comparisons with the uncoagulated (pigeon) yolks rolled on filter paper show that even the moisture values of the yolk are unmodified.

For the preparation of the white yolk samples several entire ovaries were obtained from laying hens; the ovaries were removed as soon as the birds were dead. All of the normal ova having a diameter of 3 to 6.5 mm. were separated from the ovary, cleaned, their outer theca removed, slit open, and their contents pressed out and combined in in a weighing tube. The operation is a tedious one.

The methods used in the analysis are those described in detail by Koch (5). In brief, the material was extracted four hours with alcohol, one hour with ether (then very finely pulverized), and finally twelve hours with alcohol, in a Koch extractor. Water-soluble organic and inorganic bodies were separated from both fractions resulting from the alcohol-ether extraction. The phosphorus ofthe alcohol-ether extract (exclusive of the water soluble-portion)[2] was determined by the Neumann-Pemberton method, and calculated as lecithin (phosphatides).

The figures given for neutral fat and protein were obtained by difference.[3]

CONSIDERATION OF PREVIOUS RESULTS

A single analysis by Parke (7) supplies the figures which are quoted in all text books as representing the percentage composition of egg yolk. Three yolks were combined in the sample. The moisture content as given is certainly too high, probably for the reason already noted. The extraction methods of Parke's time probably did not effect a complete separation of the lipoids. Only 68.6 per cent of the solids were alcohol-ether soluble according to Parke. In our analyses this fraction is always closer to 70 per cent.

More recently rather extensive analytical data on egg-yolk—particularly on the protein of egg yolk—have been obtained by investigators at the Connecticut State Experiment Station, and at the United States Government Laboratories. The quantity and distribution of various nitrogenous bodies have been studied in detail. Percentages of the grosser composition have however received less attention. Langworthy gives in connection with his results moisture values which are high. The yolks analyzed were from eggs obtained in the market.[4] The value of the alcohol-ether soluble fraction is slightly higher than was obtained by Parke. This author also analyzed the egg yolk of the duck, goose, turkey and guinea-fowl. Since these yolks too were from eggs obtained in the market, and hence of unknown age, the moisture values are doubtless too high. Langworthy's results upon the yolks of these species, recalculated into per cent of solids, are as follows:

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[2] This fraction from egg-yolk contains only a trace of phosphorus. In five of the analyses of the pigeon's yolk the phosphorus was determined for the entire alcohol-ether soluble fraction.

[3] Atwater and Bryant (6), state (p. 53) that "it is probable that the factor 6.25 is not correct for eggs" and that "the value of protein by difference is perhaps the more nearly correct."

[4] The object of the investigation was to learn the composition of eggs as these are obtained for food purposes.

BIRD	WATER	FAT	PROTEIN	ASH	CARBO-HYDRATES*
Duck	(45.8)	66.8	31.0	2.0	0.3
Goose	(44.1)	64.6	31.0	2.3	1.3
Turkey	(48.3)	63.6	33.6	2.3	0.8
Guinea fowl	(49.7)	63.2	33.2	2.4	0.8

* The percentages of carbohydrate are apparently based on the moist weight. The amount given for the hen's egg is 0.67 per cent.

It is stated that individual samples vary more or less and that the above figures are averages. There would seem to be real differences in the composition of yolk, for instance, from the duck and guinea fowl. It seems to us probable that if the moisture values had been obtained from ovarian, or just-laid eggs that the moisture differences would here have been—as we found in the case of the fowl and the pigeon—the most marked of all the differences. Cook (8) obtained a moisture value of 45.3 per cent from a sample containing a number of yolks, and a second value from a similar sample of 48.5 per cent. He also gives data for the ash, and for protein as calculated from total nitrogen. Pennington (9) made very extensive studies, particularly on the distribution of the nitrogen of the yolk. The moisture values given are higher than we find for the undiluted yolk; and the extract obtained with alcohol and petroleum ether is somewhat smaller than the amount we obtain.

Malcolm (10) has published some data on the percentage composition of egg yolk. For our purposes, however, the results are hardly usable for three reasons. (1) Because it seems that entire yolks were not used; and with this method of pipetting out portions of the yolk it is quite possible to include, or to fail to include, the white yolk at the center of the egg, and this will certainly introduce considerable variations—as was found by Malcolm—in the percentage of lecithin obtained. (2) The age of the eggs used is unknown. (3) The method of correcting the yolk-weight by pro-rating between yolk and albumen the loss of water from the egg before beginning analysis is wholly wrong; it magnifies instead of minimizes the error in the determination of the true initial weight of the yolk. As already noted it has been found that the yolk is constantly absorbing water from the albumen instead of losing water to the albumen as was assumed by Malcolm.

Serono and Pallozzi (11) made partial yolk analyses of fowl's eggs less than twenty-four hours old, after removing the albumen from the yolks by rolling the latter on filter paper. They did not make analyses of individual eggs but combined ten yolks in a sample. Their fat extraction seems to us inadequate, and the amount of fat obtained by them is less than that obtained by us. The amount of fat which they obtained—in spite of the large combined samples used—was also so variable as to raise much doubt as to the adequacy or accuracy of the extraction methods used. They give the minimum and maximum of fat (+lutein) obtained as (nearly) 18.87 and 21.47 per cent of the moist weight of the sample. Their figures for lecithin are, on the average (11.57 per

TABLE 1

Analyses of yolk of fowl's egg by various investigators

AUTHOR	REMARKS	MOISTURE	LECITHIN	FAT	PROTEIN	ASH
Parke	Per cent of moist weight	47.20	10.72*	22.82	15.63	0.97
Langworthy	Per cent of moist weight	49.5	33.3		15.7†	1.1
Malcolm	Per cent of moist weight		27.79 to 31.89		17.50‡	
Serono and Pallozzi	Per cent of moist weight		11.05 to 12.09	18.87 to 21.47		
Cook	Per cent of solids	45.30§ and 48.74§			30.10† and 31.87	2.11 and 2.34
Pennington	Per cent of moist weight	47.43 to 48.03¶	31.44 to 33.33			1.31 to 2.58
Riddle	Per cent of solids	47.8	20.9*	49.2	28.8	
Greenlee		47.35				

* Phosphorus of alcohol-ether extract calculated as lecithin.

† These figures = N × 6.25.

‡ This figure = the average N of 18 samples, × 6.25 (18.22, the calculated average weight of the yolks of 18 eggs).

§ The samples each contained more than one—an unnamed number—of eggs.

¶ Six to eighteen eggs used in a sample; a total of 19 analyses.

cent moist weight), slightly higher than those obtained by us. We fail to obtain moisture and protein values from their results, which were concerned chiefly with the lutein fraction of the yolk. These workers used especially elaborate precautions against the occurrence of changes in the lipoids during the analysis.

To facilitate a comparison of the results obtained by these several investigators table 1 has been prepared. The data of the table also, in some respects, amplify the foregoing brief statement. These tabulated data are moreover in convenient form for comparison with our own results. Studies similar to ours on the composition of the yolk of the pigeon's egg seem not to have been made hitherto.

DISCUSSION OF THE DATA

Yellow yolk[5] *of the common fowl.* The results of six analyses are given in table 2. An examination of the several moisture determinations shows that egg yolks obtained from the ovary and oviduct contain the smallest amounts of water. The amount is probably 2 per cent less than the figures obtained by one of us (1) and by others on "fresh" eggs. Greenlee showed that in laid eggs, kept at ordinary temperatures, solids do not pass from the yolk to the albumen. It is clear therefore that when the yolk leaves the ovary and becomes surrounded by albumen it then becomes contaminated with water absorbed from the albumen. Uncontaminated natural yolk contains little more than 45 per cent of moisture. If all of the alcohol-soluble water-insoluble phosphorus exists in the form of lecithin this substance forms 21.9 per cent of egg yolk, and the neutral fat is equal to 47.3 per cent. The amount of protein in egg yolk is 29.1 per cent.

[5] Samples of really pure yellow yolk cannot be obtained from any bird; a single full-grown ovum,—an egg yolk entire—forms each sample designated yellow yolk in table 2. At the center of each yolk there is a core—about six mm. in diameter—of white yolk; this is equal to about 1/160 of the total volume of a yolk of 33 mm. diameter. Extending from this central core to the germinal spot (or vesicle) at one point of the yolk's surface is also a cylinder of white yolk; this includes perhaps less than 1/200 of the entire volume of the egg yolk. There are furthermore five to ten quite thin strata or layers of white yolk between the central white yolk core and the periphery of the ovum; it is uncertain what proportion of the total volume of the egg is included within these strata, but probably it is as much as 3 or 4 per cent. It is clear therefore that the "yellow yolk" of our analyses is probably contaminated by about 5 per cent of white yolk The differences between the two forms of yolk are therefore somewhat greater than our analyses indicate. The eggs of White Wyandotte and Barred Plymouth Rocks were used.

TABLE 2

Percentage composition of yolk of fowl and pigeon

NO. OF ANALYSES	REMARKS	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS: Phosphatides	Neutral fat	Protein	Organic extractives	Ash	H_2O
	Yellow yolk of common fowl							
M	From ovary*	10.075	22.25	49.50	26.50	0.91	0.84	46.89*
M	M corrected	9.716	22.32	49.78	26.54			45.82
Y	First of second ovary	15.405	69.41†		30.59†	←	←	45.43
Z	Second of third ovary	8.520	18.44	49.83†	31.73†	←	←	47.17
A	From oviduct	16.20	21.90	47.26	29.10	0.56	1.19	45.38
N	From oviduct	12.822	19.15	51.88	27.23	0.86	0.83	46.99
D	Laid egg + 48 hours	17.67	20.70	49.40	28.77	0.62	0.53	47.80
	White yolk of common fowl							
R	From ova, 4.5 to 6.5 mm	6.018?‡	12.66	42.25	35.15	3.88	6.03	89.65?
48	From (93) ova 3 to 6 mm	2.688?‡	11.00	23.11	44.44	8.60	12.88	88.36?
	Yellow yolk of jungle fowl							
20	Laid + 48 hours	10.880	67.30		29.09	2.03	1.60	48.73
23	Laid + few hours	10.883	19.90	46.74	30.47	1.59	1.30	48.70
	Yellow yolk of common pigeon							
760	First + 4½ hours	3.885	20.54	69.80	30.20†	←	←	55.16
761	Second + 2 hours	3.930	20.75	68.69	31.31			54.75
886	First + 5 hours	3.272	21.67	67.65	32.35			57.17
887	Second + 5 hours	3.502	19.37	67.30	32.70			56.50
110¶	Second + 3 hours	2.813	20.15	67.55	32.45	2.00	2.11	54.21

* This, the largest yolk in the ovary of a laying hen, was covered with its inner follicular membrane. Twelve similar membranes weighed 3.110 grams and had a moisture value of about 88 per cent. The moisture figure given for this sample is therefore slightly too high for the yolk alone. Corrections made necessary by the presence of this membrane are applied in the next column.

† This figure includes ash and organic extractives.

‡ On account of the very considerable time consumed in preparing the white yolk sample it is not possible to be sure that the original moist weight is correct.

¶ Egg of wild *C. livia*.

TABLE 3

Moisture determinations (by drying at 100°C. and cooling over H_2SO_4*) of series of ovarian eggs*

	LAID EGG		FIRST OF OVARY		SECOND OF OVARY		THIRD OF OVARY		FOURTH OF OVARY		FIFTH OF OVARY	
	Weight Yolk	Per cent H_2O	Weight	H_2O	Weight	H_2O	Weight	H_2O	Weight	H_2O	Weight	H_2O
Series 1..	12.335	47.97	12.295	lost	8.520*	47.17	4.290	47.72				
Series 2..	14.9747	47.21	15.405†	45.43	12.499	45.40	8.684	45.29	5.076	45.72	1.514	48.48

* This = analysis "Z" of table 2.
† This = analysis "Y" of table 2.

Before considering the remaining sections of table 2 the moisture determinations on two series of ovarian eggs may be noted in table 3. It will be observed that those data confirm the statements made above on the smaller water content of ovarian eggs. They reflect an additional point, namely, that ova of 4 grams and less show an increase in moisture due to the relatively high proportion of white yolk in these eggs. In the following section it will become clear that this white yolk has a very high moisture value—equal to that of egg-albumen.

White yolk of the common fowl. The two analyses of white yolk give figures (table 2) which strongly contrast with the above figures for yellow yolk. One of the analyses (R) presents a less striking contrast than does the other (48). We believe this to be due, in part at least, to the fact that some ova of 6.5 mm. diameter were used in the preparation of this sample, and that such ova really contained some yellow yolk. It will be noted that *all* of the fractions from this analysis are intermediate in amount to the corresponding fraction of yellow yolk on the one hand, and to those of the less questionable sample of white yolk on the other.

It will be noted that the composition of white yolk is by no means so far removed from that of embryonic tissues as is that of the yellow yolk. The white contains but half of the neutral fats and phosphatides found in the yellow. Protein, extractives, and ash are much more abundant in the white yolk. The ratio of water to solids in the two forms shows an even greater contrast; for the yellow this ratio is, 45 : 55; for the white it is, 89 : 11.

Yellow yolk of the Jungle fowl. The Jungle fowl is usually considered the ancestor, or one of the ancestors, of the many breeds of domestic fowl. Our two analyses of yolks from Jungle fowl eggs show that in these yolks the moisture value is wholly similar to that of "fresh" eggs from various breeds of the common fowl. The amount of alcohol-soluble materials would seem however to be perceptibly less; the amount of protein appreciably greater.

Yellow yolk of the common pigeon. The five analyses here reported demonstrate a striking difference in the moisture values of the egg yolk of the fowl and the pigeon. This amount is nearly 10 per cent greater in the latter. The yolk of the common pigeon contains, moreover, a smaller percentage of water than has been found in most other species of pigeons and doves. It is therefore certain that two quite different moisture values characterize the eggs of the *Gallinae* and the *Columbinae*.[6]

TABLE 4

Averages, or most reliable percentages, of forms of egg yolk

NATURE OF YOLK	CALCULATIONS IN	WATER	PHOSPHATIDS	NEUTRAL FAT	PROTEIN	ORIGINAL EXTRACTIVES	ASH
Undiluted yolk of common fowl (yellow)	Per cent of solids[*]	45.40	20.58	49.51	28.36	(0.70)	(0.85)
	Per cent moist weight		11.15	25.25	15.04	(0.36)	(0.44)
Undiluted yolk of common fowl (white)	Per cent of solids	86.66	10.91	23.11	44.44	(3.88)	(6.03)
	Per cent moist weight		1.13	2.39	4.60	(0.40)	(0.62)
"Fresh"[†] yolk of Jungle fowl (yellow)	Per cent of solids	48.71	19.90	46.70	29.78	2.03	1.60
	Per cent moist weight		10.21	23.96	15.27	1.04	0.82
"Fresh"[†] yolk of common pigeon (yellow)	Per cent of solids	55.56	20.50	47.00[‡]	29.00[‡]	2.00	2.11
	Per cent moist weight		9.11	20.81[‡]	12.89[‡]	0.89	0.94

[*] Only in the figure for water are the "fresh-laid" eggs excluded from the averages.

[†] I.e., yolk taken from an egg within a few hours after the egg was laid.

[‡] These figures known to be approximately correct partly from data not included in table 2.

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[6] Langworthy's data for representative species of *Gallinae* support this conclusion.

The data warrant the further conclusion that the egg yolk of the pigeon contains less alcohol-soluble material, and more protein, than does the yolk of the common fowl. These figures for the pigeon more nearly approach those obtained for the Jungle fowl.

Averages and most reliable determinations. We believe that the most accurate account of the percentage composition of the various forms of yolk—as analyzed by us—cannot in all cases be obtained from the average of the several determinations. The variation of moisture due to the length of time the yolk has been in contact with albumen, and the probable contamination with traces of yellow yolk of one of the white yolk samples, are examples of probable inaccuracies that may be introduced by the 'average.'

In order to present what we believe to be the most reliable figures obtained for the various forms of yolk table 4 has been constructed. We there give also the percentages calculated in terms of moist weights in order to facilitate a comparison of our results with those of others who have presented figures based upon moist weight only. The summary form of this table will further assist the reader in making a general comparison of the gross chemical composition of the two kinds of yolk, and of yellow yolk from the three different sources.

CONCLUSIONS

The moisture values found when "fresh" laid eggs are taken for analysis are always too high. The yolk of the bird's egg begins to take up water from the albumen as soon as it leaves the ovary and enters the oviduct.

The ovarian egg (yellow yolk) of the fowl contains little more than 45 per cent of water. The solids of the fowl's egg contains 20.6 per cent of phosphatides, 49.5 per cent neutral fat, and 28.4 per cent protein.

The white yolk markedly differs from the yellow yolk in all of the six fractions studied. It is much less like "yolk" and much more like embryonic tissue in its composition. The egg-nucleus, the blastoderm and the embryo are, at all stages, in immediate contact with the white yolk—not with the yellow yolk. It is clear therefore that the medium with which these living elements are immersed is not radically different in gross composition from the living elements themselves.

The egg yolk (yellow yolk) of the Jungle fowl has probably a lower lipoid, and a higher protein content, than the yolk of domestic fowls.

The water content is probably equivalent in the (supposed) ancestral and derived forms.

The egg yolk (yellow yolk) of the pigeon differs most from that of the fowl in its much higher moisture value. Probably differences in the amount of alcohol-soluble and protein materials also exist.

The yellow yolk derived from different orders, genera and species of birds probably varies more in the amount of water than in other fractions. But the yellow yolk from birds of different orders is relatively much alike as compared with the yellow and white yolk from the same individual bird.

The extreme divergence in composition of these two forms of yolk—as deposited in the *same cell* of the individual bird—is definitely associated with two strikingly different grades of metabolism within the cell. The yellow yolk, with its very low water value, is produced when the storage powers of the cell are extremely high; the white yolk—with a water value higher than, or equivalent to, that of milk—is produced when the storage processes of the cell are much reduced.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

III. On the Metabolism of the Egg Yolk of the Fowl During Incubation

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In Liebermann's (1) very extensive study of the metabolism of the fowl's egg the entire extra-embryonic contents of the egg were combined and analyzed together. No attempt was made to follow the changes in the yolk apart from the albumen and the fluids of the amnion and the allantois. Later work has not supplied such data. The yolk and the albumen are wholly unlike substances, produced in different organs; the allantois presents during development increasing accumulations of the non-gaseous end-products of metabolism; and the amniotic fluid arises at the expense of other of the egg contents. It is therefore clear that each of the groups of substances runs a separate course in the developing eggs and that the picture obtained from a study of all of them combined may very imperfectly reflect the course of events in any one of the group. The present study deals with changes in the *yolk* during incubation. Our chief problem was to determine the relative rates at which the various constituents of yolk are removed and utilized by the embryo.

Such a study requires the isolation and chemical analysis of the yolks of eggs subjected to various periods of incubation. The clean and perfect isolation of yolk samples from the incubating egg offers a number of difficulties, some of which it is necessary to describe. (1) At all stages the presence of a certain amount of living tissue—the yolk-sacs—offers a complication. It would be difficult to obtain all adhering traces of yolk from the yolk-sac, and since considerable quantities of yolk are met with in large intracellular spaces (intracellular yolk) of the sac it seems highly desirable to analyze the yolk-sacs along with the unabsorbed yolk of the various stages of incubation. But this combination of living tissue with unmetabolized yolk-residues requires additional and separate analyses of the yolk-sac tissues of the various stages being studied, in order that one may know how much and in what direction the presence of this tissue affects the figures obtained for the yolk analyses. (2) The yolk-sac completely encloses the yolk on the twelfth day of incubation. Previous to this stage the vitelline membrane at the lower pole of the egg is so fragile as to make the separation from albumen an uncertain and difficult task. We have therefore confined our study to periods beginning with the twelfth day. (3) Even after completely covering the yolk the yolk-sac continues to grow. During this same period the amount of yolk rapidly diminishes. The relative proportions of these two materials therefore steadily changes during incubation. (4) More or less solid and viscous *bodies* were found in a fraction of the yolk-sacs of very late stages of incubation. Such bodies are surrounded by a much more fluid yolk. The presence of one or more of these bodies in an analysis of yolk-sacs plus their undigested residues will very considerably influence the results; for, two such bodies separately analyzed (41 and 35 of table) differ quite markedly from all other forms of yolk at a corresponding stage of incubation. We are not sure that such bodies were absent from all of the analyses of yolk-residues of intact yolk-sacs.

The methods of analysis were those of the preceding paper of this series of studies (2). A full description of the methods has been given by Koch (3). The phosphorus of all of the analyses was calculated as lecithin (phosphatides).

THE YOLK-SAC AND ITS CHEMICAL COMPOSITION

The yolk-sac of the chick is a highly vascular, glandular, entodermal (splanchnopleure) membrane which by the twelfth day of incubation has completely surrounded the yolk; it has also separated with approximate completeness the yolk from the remnant of egg albumen. The twelve day stage was for this reason selected as the first stage for chemical study. Fully formed membranes and clean samples of the contained yolks may both be obtained at this period. The yolk-sac with its residue of yolk is stated by Virchow (4) and others to be drawn within the body of the chick usually on the nineteenth day of incubation, but to reach its highest development a little later,[1] and to maintain its highest development to at least a day or two after hatching. Yolk-sacs of the nineteen day stage were therefore selected as the second or later stage for study. In collecting the samples, however, it was observed that the yolk-sacs sometimes remained outside of live embryos of even twenty-one days of incubation; a few samples of twenty and twenty-one day stages were therefore also collected[2] and analyzed.

From the preceding statement it is evident that there is no reason for identifying any one of these stages of the yolk-sac with greater or less *activity* than another stage.[3] In accord with the inference that the two stages are functional equivalents, the analytical data from the samples of twelve and nineteen day membranes (45 and 32 of composite table) are rather closely similar. The twenty-one day preparation (F) was much smaller than the preceding, and unlike those samples was not freed from "intra-cellular" yolk. For these reasons it is improbable that the apparent divergence in the composition of this stage from earlier stages is real. The differences are those to be expected from our failure completely to free this sample of yolk.

The three stages of this membrane were analyzed not primarily for comparison with each other, but for the purpose of a check upon the figures obtained for the analyses of the yolk-sacs plus their unassimilated residues of yolk.[4] The ratio of phosphatides to neutral fat in the yolk-sac tissue will be seen (first section of table) to be somewhat higher than in egg yolk; the ratio of the former being about 7 : 9; of the latter 2 : 5. Nearly 75 per cent of the yolk-sac tissue is protein.

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[1] A comparison of the weights of twelve day and nineteen day yolk-sacs whose combined weights are given in the table (45, 32) plainly shows that the latter are much larger. The increase in size of the yolk-sac after the time of complete enclosure of the yolk (twelve days) is chiefly due to the extension of the folds or septa of the inner wall. The dimensions of the external boundary of the yolk-sac are probably little changed during this period.

[2] Large numbers of eggs of white Wyandottes were incubated to appropriate stages. Where yolk-sacs free from their yolk contents were wanted the sacs were freed from albumen and umbilical connections and rinsed rapidly in physiological salt solution followed by dipping in distilled water and then removing the surplus moisture with filter paper. The sacs were then slit entirely open and the interior flushed and cleaned in a similar way. There remained, particularly in the eggs incubated beyond the twelve day stage, evident collections of "intra-cellular yolk" within the walls of the yolk-sac. These points were punctured and all such visible yolk was pressed out and flushed with the cleansing fluids. Where the yolk-sacs plus the contents were used only the external cleansing was required. For the several samples of fluids or bodies taken from within yolk-sacs no special treatment whatever was necessary.

[3] The yolk-sac is actively functioning and actively growing in both the twelve and nineteen day stages.

YOLK NOT METABOLIZED BY CHICK EMBRYOS OF 12, 18, 20, AND 21 DAYS

The results of analyses of yolk of these four stages are given in the second and third sections of the Composite Table. A yolk of twelve days incubation (26) shows that the proportions of lecithin, neutral fat and protein remain practically as they were in the fresh yolk. The proportion of water has been increased about 9 per cent. The amount of these substances in pure unmodified yolk was shown by Spohn and Riddle (2) to average as follows: Lecithin, 20.58 per cent; neutral fat, 49.51 per cent; protein, 28.36 per cent; water 45.40 per cent. The yolk of twelve days incubation yielded 20.07 per cent lecithin; 48.60 per cent of neutral fat; 27.56 per cent protein; and 54.76 per cent of water. There is little doubt that up to the twelfth day of incubation the various solids of the yolk are removed from it in not very unequal proportions.

Between the twelfth and eighteenth days, however, the lecithins and fats are mobilized to a greater extent than the proteins. These facts are plainly shown by analyses *G* and *H* (see table). Our data show further that during this period the proportion of water has suffered a reduction of nearly 9 per cent, i.e., to the normal proportion for unmodified yolk. In connection with these data for twelve day and 18 day incubations reference may be made to a single analysis by Parke (5) of a yolk incubated for ten days, and of another incubated for seventeen days. Unfortunately Parke fails to say anything whatever of the preparation of these samples, and his extraction certainly failed to separate all of the fat. His moisture determinations, however, are doubtless of value. In the ten day egg this was found to be

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[4] Six such analyses are reported in the second section of the table. An examination of the weights of these samples will show that the amount of membrane present in each is almost negligible for some of the determinations. The data are so placed in the tabulation, however, that the direction in which the living tissue influences the figures for the entire sample need in no case be wholly overlooked. Estimates of the proportion of membrane present in each of these analyses are given in the form of a fraction placed in parentheses immediately underneath the moist weight of the several samples.

Composite table

	NATURE OF TISSUE	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS					H_2O
			Phosphatides P. as lecithin	Neutral fat	Protein	Organic extractives	Ash	
45	Yolk-sacs (41) 12–14 days incubation	1.055?	7.47	8.23	74.82	4.06	5.42	74.56
32	Yolk-sacs (13) 19–20 days incubation	1.150+	7.17	10.25	74.52	3.64	4.42	70.40
F	Yolk-sacs (3) 21 days*	0.482	trace	29.09	65.06	3.33†	2.52†	74.48
26	Yolk-sac (J. F.) and contents‡ 12 days incubation	11.090 (1/120)§	20.07	48.60	27.56	2.02	1.75	54.76
G	Yolk-sacs (3) and contents 18 days incubation	34.096 (1/60)	16.81	45.67	37.86	0.45†	0.23†	45.68
H	Yolk-sacs (3) and contents 18 days incubation	35.275 (1/60)	13.25	38.78	46.73	0.69†	0.53†	46.48
35	Yolk-sac and contents 20 days incubation	13.775 (1/40)	10.82	26.73	59.61	1.61	1.23	52.09
36	Yolk-sac and contents 20 days incubation	15.448 (1/40)	14.74	38.19	44.86	1.08	1.13	47.75
E	Yolk-sacs (3) and contents 21 days incubation	9.450 (1/15)	17.62	47.26	32.73	1.39	1.16	56.52
37	More liquid contents from yolk-sac (20 days)	4.262	11.76	33.09	51.88	1.96	1.31	44.17
39	More liquid contents from yolk-sac (20+ days)	4.624	11.23	30.02	55.25	2.00	1.49	54.18
41	Central yolk body (less viscous) of 20 days yolk-sac	0 800	19.68	46.05	28.87	2.00	3.40	37.13
34	Central yolk-body (quite viscous) of 19 days yolk-sac	2.532	3.95	8.71	82.60	2.23	2.51	71.27
33	Intracellular yolk and yolk-sac at 19 days	2.280 (1/10)§	8.91	64.83	18.80	4.27	3.19	78:84

Composite table—Continued

	NATURE OF TISSUE	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS					H₂O
			Phosphatides P. as lecithin	Neutral fat	Protein	Organic extractives	Ash	
0	Resorbed ovum (from hen)	1.834	15.30	45.65	35.26	2.08	1.71	63.20
5¼	"Extra foll. yolk" (fowl).	1.605	19.05	45.49	26.21	3.47	5.78	74.22

* Not really freed from fat; the "intracellular" yolk not pressed out.

† The figure is for the alcohol-soluble substance only.

‡ This and following five analyses were of yolk-sacs and their contents entire.

§ The fractions of this column are estimates of the amounts of living tissue (sac) present.

We are under obligations to Miss Adelaide A. Spohn for assistance with this series of analyses.

10 per cent higher than in "fresh yolk;" in the seventeen day egg the moisture was reduced nearly 13 per cent from the ten day level, or nearly 3 per cent below that of fresh yolk. In general, this result accords with our own data on the fluctuations in the moisture value during this period.

The data for the yolk residues attached to twenty day embryos show (35, 36) that the preponderant utilization of lecithin and fat is continued to this stage. The amount of these substances being proportionately still further reduced, while the relative amount of protein is still further increased. The amount of water in this and the twenty-one day stages again unmistakably increases. The relative amount of protein in the twenty day stage is nearly double that present in fresh yolk; the proportion of phosphatides[6] is slightly less than three-fifths that found at the beginning of development; the relative amount of neutral fat present is a little more than three-fifths of the amount originally present. In all (seven) of the analyses of eighteen to twenty-one day stages the phosphatides show a somewhat greater reduction than do the neutral fats.

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[6] Liebermann did not separate or determine the phosphorus or phosphatides in his analyses; his figures for protein apply, as previously noted, to all the extra-embryonic contents of the egg—not to the yolk alone; and the proportion of ether-soluble substance cannot be stated or followed, as we have done above, because the original weight of yolk-substance alone was undetermined, as was also the amount of extraneous protein which formed a part of his "total solids." We are therefore unable to use any of Liebermann's data in the comparisons made above.

Two analyses (37, 39) of yolk that could be poured from slitted yolk-sacs of twenty, and twenty and one-half days incubation fully confirm the above findings on the relative proportions of yolk subtsances at this stage. The phosphatides here average 11.5 per cent; the neutral fat 31.5 per cent; the protein 53.5 per cent. The moisture values here are doubtless somewhat too low since this yolk was directly exposed to the air while being collected from the sacs and weighed. The figures are 44.2 per cent and 54.2 per cent and here again the egg that had gone somewhat beyond the twenty day stage has the considerably higher moisture value.

The single analysis (E) of yolk of twenty-one days incubation shows only a part of the considerable reduction of phosphatides (17.6 per cent) noted in the immediately preceding stages. Moreover, the neutral fat (47.3 per cent) is only slightly less than that found in fresh yolks, and the amount of protein (32.7 per cent) only a little higher. Attention may, however, be called to the fact that the amount of yolk in the yolk-sac of this stage was quite small in comparison with the amount present in the eighteen and twenty day sacs; and further that it is unusual (abnormal?) to find the yolk-sac still external to the embryo—as these were—after the twentieth day of incubation.

Our data indicate that in the period during which the greater part of the yolk is mobilized—the twelfth to the twentieth day of incubation—the phosphatides are metabolized more rapidly than other substances. The neutral fats are utilized more rapidly than the proteins; this holds true for the eighteen to twenty day period, in which the protein of the yolk is the sole source of protein for the embryo.[6] The amount of moisture in the yolk increases to, or beyond, the twelve day stage; between the twelve and eighteen day stages it is decreased; it is probably again increased at or near the very end of yolk absorption.

UNUSUAL FORMS OF YOLK—AND YOLK METABOLIZED IN SITUATIONS OTHER THAN IN YOLK SACS

Reference has already been made to the occasional presence of semi-solid or viscous bodies in yolk-sacs at the close of incubation.

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[6] The egg albumen which surrounds the yolk entirely disappears at about the sixteenth or seventeenth day of incubation.

to be wholly different in their composition. The smaller, less viscous Two such bodies were analyzed (see 41 and 34 in table) and proved body from a yolk-sac of twenty days incubation, was practically normal unchanged yolk; little changed except in its moisture value which was about 13 per cent too low for yolk of this stage of incubation, and the lowest thus far found in any form or stage of yolk.

The larger more viscous yolk-body was removed from a yolk-sac of nineteen days incubation. In its composition it bears much less resemblance to normal yolk than to the living tissue of the yolk-sac. This fact becomes quite plain by reference to the table. The moisture value of this body was extraordinarily high—71.3 per cent. This value exceeds by more than 15 per cent the moisture of any other form of yolk which we have obtained from *within* a yolk-sac.

The fact that in late stages of incubation we sometimes meet with bodies so unlike chemically as the two just described is certainly a fact of importance in any chemical study of the yolk-residues of this period. In the later samples collected by us the sacs were manipulated between the fingers to assure ourselves of the absence of these semi-solids. The origin of these bodies is quite unknown.[7]

Yolk which has passed through the innermost layer of cells of the yolk-sac epithelium is found in masses of considerable size at many points on yolk-sacs of late incubation stages. It is of interest to know the composition of such quasi-mobilized yolk. The single analysis (33) we have made of this substance indicates that it is a highly modified yolk. The phosphatides (8.9 per cent) are not quite half that of normal yolk. Curiously, however, the proportion of fat is considerably higher (64.8 per cent) than that of normal yolk. The relative amount of protein is only two thirds (18.8 per cent) that of fresh yolk and less than half the amount of protein of yolk-residues of this stage of incubation.

Yolk is sometimes formed in a situation quite outside of eggs as was shown by me in an earlier publication (6). Yolk thus formed, and collected from free spaces of capsules which have liberated ova, is shown by a single analysis (57) given in the table, to be true yolk. The moisture value of this yolk is however extremely high, namely 74.2 per cent. The slightly lower percentage of phosphatides, fat and protein is probably accounted for by the abnormally high ash and extractive figures. These latter figures are probably too high because of the smallness of the sample.

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[7] One may conjecture that those viscous bodies which have a composition approximating that of pure yolk were formed by a rather prolonged and complete *halting* of the growth of the egg when it had attained a diameter of perhaps 10 mm. or 12 mm.; and that normal yolk deposition was later resumed about this central modified core (most of the eggs used for this work were summer eggs, and the birds were laying by no means regularly). It is perhaps conceivable that the bodies of the type which differs so radically from yolk, are pinched-off septa of the inner wall of the yolk-sac which are later infiltrated with traces of yolk; or, that they represent the final clump of egg-albumen driven into the yolk and partly infiltrated with it.

In those cases in which nearly full grown ova are resorbed by the follicle which surrounds them, it is possible to study in a totally different situation the relative rates at which the constituents of yolk are mobilized. Only one analysis of such yolk has been made. This furnishes, however, a very interesting parallel to the results obtained from the above described studies on yolk of the yolk-sac. Here again (see analysis *O* of table) the phosphatides were removed most rapidly; the neutral fats next most rapidly, and the proteins slowest of all. The moisture value (63.2 per cent) here also is much increased.

CONCLUSIONS

The composition of the yolk-sac of the fowl was determined for the twelve and nineteen day stages of incubation.

A differential utilization of the elements of yolk prior to the twelfth day of incubation has not been shown to occur.

A study of the unmetabolized yolk of twelve, eighteen and twenty day stages shows that after the twelfth day the phosphatides are utilized more rapidly than the neutral fats. And the neutral fats are utilized faster than the proteins. This order of utilization of these substances persists during the eighteen to twenty day period when the embryo's sole source of protein is the protein of the yolk.

The moisture value of the yolk undergoes very considerable fluctuations during incubation. At the twelfth day it is about 9 per cent higher than in fresh undiluted yolk. At the eighteenth day it has fallen to near the value for fresh yolk. At the very end of incubation this value probably rises considerably.

Two forms of semi-solid yolk bodies which are occasionally present in final stages of incubation were found to be wholly unlike in their chemical composition.

Yolk resorbed by the follicle which secreted it shows—like yolk mobilized from the yolk-sac—a more rapid utilization of the phosphatides (lecithin); the neutral fats are utilized at a somewhat slower rate; while the proteins are metabolized more slowly than either the phosphatides or neutral fats.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

IV. When a Gland Functions for the First Time is its Secretion the Equivalent of Subsequent Secretions?

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The question of differences in composition of the initial secretion of a gland, as compared with subsequent secretions from the same gland, seems hitherto not to have been investigated. The nearest approach to information of this kind concerns the product of the mammary glands. It is obvious, however, that it is not possible to study the very first secretion of these glands since they function to a greater or less extent at or near the time of birth, and this earliest secretion is too small in amount, as well as too involved in time relations, to permit a quantitative analysis. Moreover few if any of the glands of the mammalian body can be utilized for such a study.

In the albumen-secreting gland of the bird's oviduct, however, it is easily possible to know when the gland *first* secretes albumen, since this coincides with the time of liberation of the first ovum from the ovary and the time of laying of the first egg. Too, the amount of the secretion is here sufficient for chemical analysis. The egg-albumen of the common pigeon has here been used because, as a matter of routine, in our collection of these birds we keep accurate records of the date of laying the first egg in life.

METHODS OF PREPARATION AND ANALYSIS

The samples of egg-albumen for analysis were taken from eggs immediately after laying—in one-half hour to two hours after laying. This is important since we have found (No. 2 of these studies) that the egg-yolk absorbs water from the albumen, in addition to the well known continuous loss of water from the albumen to the air. The perfectly fresh eggs—before any trace of air space begins to form—are weighed, steamed for eight minutes, and the shell carefully removed without disturbing the shell membrane of the egg. This shell membrane forms a part of the albumen sample. The hardened yolk is then removed and weighed at once. The weight of the yolk, plus that of the shell is subtracted from the original weight of the entire egg, and this gives the true weight of the albumen sample.[1] The albumen is preserved at once in absolute alcohol.

The methods of analysis are those described by Koch[2]—a four-hour alcohol extraction, followed by a one-hour ether extraction (after which the material is very finely pulverized), and this by a twelve-hour alcohol extraction. The ash was separately determined for the alcohol-ether-soluble, and alcohol-ether-insoluble fractions.

EXAMINATION OF THE DATA

Thirty samples of egg-albumen were analyzed. These include samples obtained from the first eggs in life, obtained both in summer and winter;[3] the immediately next few eggs from these same birds were analyzed, and after an interim still other eggs were taken from the same birds. For further comparison, samples were taken from older birds—birds that had previously laid large numbers of eggs. These samples were likewise taken in both winter and summer.

The results are fully given in table 1. It is clear from an examination of these data that the albumen from the first and immediately succeeding eggs of the pigeon contains measurably less water, and more alcohol-ether-insoluble substance, than does the albumen secreted later by these same birds; and that these earliest secretions bear a similar relation to the later secretions of other (older) birds as well.

The first five eggs in life of ♀ 545 show an average water content of the albumen of 89.08 per cent. The average of four of the later eggs of this same bird (her 23d to 26th) was 89.61 per cent. The averages of eggs laid by other birds after numerous previous eggs are: (♀ A421) 4 = 89.47 per cent, 2 = 89.93 per cent; (♀ A410) 4 = 89.47, 2 = 89.25 per cent.

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[1] The removal of the shell is a rather tedious process and permits an evaporation of water which prevents the direct weighings of the albumen.

[2] Koch: The Jour. of the Amer. Chem. Soc., xxxi, no. 12, 1329.

[3] Certain differences which one of us (R.) have found to obtain in the size of yolks from summer and winter eggs (or rather spring and autumn) led us to carry the observations over the two seasons.

The albumen of the first five eggs of ♀ 511 averages 89.12 per cent water. Two later eggs (her 23d and 24th) average 89.33 per cent water, More significant than the comparison of the averages of the figures obtained from these groups of earliest eggs with groups laid later is the comparison of the values obtained for the individual eggs of the two groups of earliest eggs. There are two such groups (♀'s 545, 511) of five eggs each. In both groups the first pair in life are smaller than the succeeding pair—also the smallest of the entire series of analyses—and in both groups, too, the fifth egg in life has a higher moisture value than either of the four previous eggs. Moreover, the four eggs of the initial pairs of the two groups, all show a lower moisture value than does any egg laid by these birds in their second period, and also lower than any egg laid by birds which had previously laid numerous eggs.

A pair of eggs—first in life—from a third female (A 105) gave a moisture value for the albumen of 89.27 per cent. This is a somewhat higher value than that obtained from the other first pairs in life, but is again less than the average for any bird that had previously laid eggs.

The differences in the moisture values are indeed not large, but they are so consistent as to leave little doubt of their reality. Next in interest, perhaps, to the fact of differences in first and later secretions of the gland, is the fact of the smallness of the difference. If—in the terms of another science—a gland "learns" by doing, it "learns" but little and the initial effort of this gland is in many respects a remarkable approximation to its later and most practiced efforts.

Whether there are differences in the percentages of alcohol-ether-soluble substance, and of ash in the two fractions, is perhaps not definitely answered by the data. But no differences are consistently indicated by the figures. The increase in solids is found in the alcohol-ether-insoluble fractions.

Neither season nor the variable amounts of albumen and yolk present in the various eggs seems to have any consistent influence on the percentage of water in the albumen.

CONCLUSIONS

It has been possible to compare the results of the very first functionings of a gland, with later functionings of the same gland.

The albumen secreted by the pigeon's oviducal glands, in their initial functionings, is not the exact quivalent of albumen secreted

TABLE 1

The chemical composition of egg albumen (pigeon) from the first, and from later, functionings of the oviduct

Winter season

First functionings of oviduct

NO. OF SAMPLE (ALBUMEN)	NO. OF BIRD	NO. OF CLUTCH (IN LIFE)	DATE OF LAYING	WEIGHT OF YOLK	WEIGHT OF SHELL	WEIGHT OF ALBUMEN	PER CENT H_2O	PER CENT ALCOHOL SOLUBLE	PER CENT ALCOHOL INSOLUBLE	PER CENT ALCOHOL SOLUBLE ASH	PER CENT ALCOHOL INSOLUBLE ASH
779	545	1^{1*}	December 6, 1914	2.900	1.005	12.445	88.93	0.77	10.30	0.33	0.35
780	545	1^2	December 8, 1914	2.910	1.080	12.360	88.88	0.80	10.32	0.34	0.32
795	545	2^1	December 24, 1914	3.280	1.030	13.050	88.91	0.79	10.30	0.35	0.34
804	545	3^1	January 4, 1915	3.165	1.020	13.165	89.06	0.81	10.13	0.34	0.38
805	545	3^2	January 6, 1915	3.560	1.085	12.625	89.62	0.86	9.52	0.35	0.33
Average				3.147	1.044	12.609	89.08	0.81	10.11	0.34	0.35
778	511	1^2	December 3, 1914	2.860	0.981	12.799	89.00	0.80	10.20	0.32	0.36
783	511	2^1	December 12, 1914	2.935	1.035	13.230	88.79	0.68	10.53	0.33	0.36
784	511	2^2	December 14, 1914	3.150	1.000	13.305	89.24	0.73	10.03	0.34	0.32
793	511	3^1	December 23, 1914	2.960	0.960	12.955	89.03	0.72	10.25	0.33	0.35
794	511	3^2	December 25, 1914	3.182	1.002	13.338	89.36	0.70	9.94	0.33	0.36
Average				3.017	0.996	13.125	89.12	0.73	10.19	0.33	0.35

Later functionings of oviduct (of other individuals)

NO. OF SAMPLE (ALBUMEN)	NO. OF BIRD	NO. OF CLUTCH (IN LIFE)	DATE OF LAYING	WEIGHT OF YOLK	WEIGHT OF SHELL	WEIGHT OF ALBUMEN	PER CENT H_2O	PER CENT ALCOHOL SOLUBLE	PER CENT ALCOHOL INSOLUBLE	PER CENT ALCOHOL SOLUBLE ASH	PER CENT ALCOHOL INSOLUBLE ASH
829	A421	$+^{1}\dagger$	February 20, 1915	3.360	1.070	12.320	89.33	0.89	9.78	0.37	0.28
830	A421	$+^2$	February 22, 1915	3.750	1.170	13.260	89.44	0.88	9.68	0.38	0.28
838	A421	$+^1$	March 2, 1915	4.020	1.130	13.805	89.48	0.87	9.65	0.38	0.28
839	A421	$+^2$	March 4, 1915	4.100	1.240	13.630	89.64	0.95	9.41	0.39	0.26
Average				3.808	1.153	13.254	89.47	0.90	9.63	0.38	0.28
831	A410	$+^1$	February 21, 1915	3.830	1.140	13.210	89.35	0.89	9.76	0.37	0.30
832	A410	$+^2$	February 23, 1915	3.990	1.120	13.190	89.53	0.88	9.58	0.38	0.31
836	A410	$+^1$	March 2, 1915	3.220	1.095	12.745	89.62	0.90	9.47	0.38	0.27
837	A410	$+^2$	March 4, 1915	3.620	1.090	13.435	89.38	0.87	9.75	0.37	0.29
Average				3.665	1.111	13.145	89.47	0.89	9.64	0.38	0.29

Summer season

First functionings of oviduct

938	A105	1^1	July	15, 1915	2.990	1.000	11.220	89.22	0.77	10.02	0.30	0.57–‡
939	A105	1^2	July	17, 1915	2.598	1.025	11.400	89.32	0.45	10.23	0.25	0.35
Average					2.794	1.013	11.310	89.27	0.61	10.13	0.28	0.35

Somewhat later functionings of oviduct (see above)

923	545	12^1	June	25, 1915	3.240	0.975	14.595	90.61	0.62	8.78	0.34	0.27
924	545	12^2	June	27, 1915	3.380	1.060	13.955	89.03	0.66	10.31	0.33	0.28
927	545	13^1	July	5, 1915	3.532	1.095	14.310	89.60	0.66	9.74	0.32	0.33
928	545	13^2	July	7, 1915	3.620	1.120	14.067	89.19	0.64	10.16	0.30	0.28
Average					3.443	1.063	14.232	89.61	0.65	9.75	0.32	0.29
940	511	12^1	July	19, 1915	3.970	1.060	14.310	89.27	0.73	10.00	0.38	0.34
941	511	12^2	July	21, 1915	3.857	1.060	13.401	89.38	0.73	9.89	0.35	0.31
Average					3.914	1.060	13.856	89.33	0.73	9.95	0.37	0.33

Later functionings of oviduct

925	A421	$+^1$	July	1, 1915	4.370	1.190	13.650	89.85	0.70	9.45	0.35	0.32
926	A421	$+^2$	July	3, 1915	4.595	1.295	13.975	90.01	0.71	9.27	0.35	0.27
Average					4.441	1.243	13.813	89.93	0.71	9.36	0.35	0.30
942	A410	$+^1$	July	19, 1915	3.394	1.075	11.171	89.33	0.81	9.86	0.39	0.35
943	A410	$+^2$	July	21, 1915	3.462	1.060	11.733	89.17	0.86	9.97	0.37	0.59‡
Average					3.428	1.068	11.452	89.25	0.84	9.92	0.38	0.35

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* The exponents of this column indicate that particular eggs were *first* or *second* of the clutch.

† The plus (+) sign indicates that this bird had previously laid *numerous* eggs.

‡ This figure probably inaccurate.

later by these glands. The difference concerns chiefly the relative proportions of water and solids.

Albumen produced in initial efforts contains a smaller percentage of water and a higher percentage of alcohol-ether-insoluble substance.

During a few, or several, of the earlier functionings of the oviducal glands, there probably occurs a *gradual* change from the initial lower percentage of water to the later higher percentage of water.

Partial analyses of the albumen of the pigeon's egg are recorded and one source of variation in the moisture value of this albumen has been identified.

The amounts of alcohol-ether-soluble substance, and of inorganic matter probably do not vary widely nor consistently.

The amount of water in the egg albumen of the pigeons studied shows no consistent variation in respect to summer and winter.

The earliest secretion of the albumen-secreting gland of the pigeon's oviduct is, in several respects, a rather close approximation to the later products of the gland.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

V. THE EFFECT OF ALCOHOL ON THE SIZE OF THE YOLK OF PIGEON'S EGG

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It is known[1] that normally in pure species of doves and pigeons a difference in size usually exists between those ova (yolks) which will prospectively produce males and females; the males more often arise from the smaller, the females from the larger ova. One of us proposed to study the effects of alcohol and other anaesthetics on sex development and control, and it seemed advisable to carry on parallel studies on the effects of some of the anaesthetics on the size of the eggs. The results here recorded are those thus far obtained with alcohol.

These data show that the ova produced with a certain amount[2] of alcohol regularly present in the blood for a considerable part of the day are smaller than the ova normally produced by the same bird. This result, and indeed any result whatever would, perhaps, have been difficult to foretell. The normal growth of the yolk (or ovum) during its last five days in the bird's ovary is made at an exceedingly rapid rate—as shown in number 1 of these studies[3]—and the materials for this growth are of course derived from the blood in competition with the other tissues of the body. Whether alcohol—which certainly modifies the metabolic rate of the tissues, and is easily soluble in the egg-yolk—will in the sum of its effects in the organism facilitate or hinder the storage processes in the egg, was the problem of particular interest to us. No data seems hitherto to have been obtained bearing on the question. Niclaux[4] and Renault have shown that alcohol has a decided affinity for the reproductive glands, even of mammals, where the proportion of fatty substance is quite small as compared with the functioning ovary of the bird. And Stockard[5] has demonstrated that alcohol influences the hereditary capacities of the treated germs of mammals.

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[1] Riddle. Sci., N. S., xxxv, 462; Bull. Amer. Acad. of Med., xv, No. 5, 265; Amer. Nat., 1916, l, 285.

[2] Only the particular dosage used in these experiments can now be considered; it is not improbable that a dosage producing an opposite effect will later be found.

[3] Riddle: This Journal, 1916, xli, 387.

Although the "alcoholized germs" from this series were smaller than the normal—thus resembling in size the more typical male-producing eggs—they were nevertheless probably "weaker germs"[6] than the normal. Incubation tests of several of the eggs of females 624 and 631 indicate that both of these birds produced under alcohol a predominance of females. For the reasons stated in the preceding paragraph it is not permissible to say that the smaller size of eggs produced under alcohol is an evidence of their being made more like male-producers. The size attained by the ovum under alcohol is the expression of a new metabolic adjustment between ovum and competing somatic tissues under the influence of an added factor. The smaller of the "alcoholized" eggs are—as is the case among the normal ones—probably prospectively more masculine than the larger "alcoholized" eggs. But size comparisons cannot be made directly from an alcoholized series to an unalcoholized series, since the planes or levels of body metabolism under which they were produced were probably quite different.

METHODS AND DOSAGE

The alcohol was given by inhalation.[7] The birds were placed in a glass box of 6 cubic feet capacity; this box afforded slight ventilation about the edges of the sloping lid or top (forming upper part of side as well as top), and had a ⅜ inch opening at either end on a level with the floor. About 2¼ square feet of alcohol-pan surface was exposed. The amount of surface available for the evaporation of alcohol (95 per cent) into the air of the tank was further much increased by the use of large amounts of cotton-batting projecting above the surface of the alcohol. In a preliminary trial the group of birds to be tested was left in the tank without alcohol for more than five hours without showing any discomfort; with alcohol in the tank locomotion became quite unsteady or impossible in two to three hours. The period of alcoholization lasted usually one, one and one-half to two hours. With the exception of March 22, March 28 to April 2, April 7 and May 17 they were dosed daily. Four of the birds were treated for four months; three for only two months.

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[4] Niclaux: L'obstetrique, 1900, Tm. xv.

[5] See note 7.

[6] This fact was established by other studies on the eggs of this "alcohol series" which were incubated, instead of opened for the measurement of the yolks.

[7] This method has been described by Stockard, Arch. Int. Med., October 1912, and Arch. f. Entw'mech., 1912, xxxv, 3.

The females of various kinds of doves and pigeons were selected for the test—two common pigeons (*C. livia dom.*) two blond Ring doves (*St. risoria*) and three hybrids. The weights of the yolks produced by these birds immediately preceding alcoholization had been learned. Eggs laid during the alcoholization were steamed (as were the earlier ones) for eight minutes to harden the yolks which were then removed and weighed. A considerable number of eggs produced during this period were tested for fertility, longevity and sex and will not be further treated here. The yolks of several eggs, laid after the period of alcoholization was over, were taken from each of the treated females and their weights ascertained.

DISCUSSION OF THE DATA

The weights were taken of 114 yolks. Of these, 31 are from the pre-alcoholization, 45 from the alcoholization, and 38 from the post-alcoholization period. The results are presented in table 1. It will be seen that six of the seven alcoholized birds show a very notable decrease in size of yolks produced under alcoholization. The seventh (no. 384) showed a very slight increase in size. This bird was one of three alcoholized for the shorter period—only two months. In six cases also the yolks of the post-alcoholization period were notably smaller than those of the pre-alcoholization period.[8] In the single exception (no. 242) the difference between the two is slight. When a further fact[9] is taken into consideration, namely that during the progress of the season, i.e., during the alcoholization and post-alcoholization periods—the yolks of untreated birds normally become *larger*, the significance of the figures given in table 1 cannot be questioned.

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[8] The ova were exposed to the effect of the alcohol while they were in their period of slow growth (see no. 1 of this series of papers), but not during the final period of four to five days of rapid growth as were those of the alcoholization period.

[9] See literature cited under note 1. The weights of the treated birds did not decrease during the alcoholization period.

TABLE 1

Effect of alcohol (March 17 to July 17, 1914) on size of egg

	♀242. Hybrid*	
Average of 4 eggs, January	4 to February 18, before alcohol period..	1.921
Average of 4 eggs, April	7 to May 8, during alcohol period..	*1.744*
Average of 8 eggs, May	18 to December 19, after alcohol period..	1.956
	♀290. Hybrid*	
Average of 4 eggs January	13 to February 12, before alcohol period..	1.915
Average of 4 eggs, March	17 to May 5, during alcohol period..	*1.776*
Average of 8 eggs, May	23 to September 15, after alcohol period..	1.710
	♀624. Blond Ring	
Average of 5 eggs, January	12 to March 14, before alcohol period..	2.225
Average of 4 eggs, March	31 to April 13, during alcohol period..	*2.110*
Average of 5 eggs, July	21 to November 2, after alcohol period..	2.072
	♀631. Blond ring	
Average of 4 eggs, January	28 to March 11, before alcohol period..	1.813
Average of 10 eggs, March	30 to July 17, during alcohol period..	*1.758*
Average of 11 eggs, July	23 to December 29, after alcohol period..	1.793
	♀646. Common pigeon	
Average of 4 eggs, January	18 to March 11, before alcohol period..	4.003
Average of 5 eggs March	20 to July 17, during alcohol period..	*3.209*
Average of 3 eggs, September	25 to November 26, after alcohol period..	2.603
	♀(102). Common Pigeon	
Average of 6 eggs, February	11 to March 11, before alcohol period..	3.728
Average of 10 eggs, March	21 to July 4, during alcohol period..	*3.356*
Average of 1 egg, August	19, after alcohol period..	3.155
	♀384. Hybrid†	
Average of 4 eggs, February	10 to March 5, before alcohol period..	1.343
Average of 8 eggs, March	21 to May 18, during alcohol period..	*1.358*
Average of 2 eggs, July	9 to July 11, after alcohol period..	1 145

* Alcohol discontinued May 16, 1914.

† Alcohol discontinued May 17, 1914.

CONCLUSIONS

When alcohol is given by inhalation for one to two hours daily, during a period of months, to doves and pigeons the yolks produced by these birds become smaller than during the pre-alcoholization period. This decrease occurs even during the season when the yolks of untreated birds normally grow larger than in the earlier period.

Yolks produced during a few weeks or months after the alcoholization period are smaller than normal.

It cannot be inferred that the reduction in yolk size which occurs under alcoholization renders these eggs more like the smaller eggs of the untreated doves in their *developmental capacities*. Size of yolks in the two groups is not directly comparable on this basis, since the alcohol introduces an important added factor. The evidence at hand indicates that though the alcoholized yolks are reduced in size, they are developmentally more like the larger normal eggs, than they are like the smaller normal eggs.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

VI. SEXUAL DIFFERENCES IN THE FAT AND PHOSPHORUS CONTENT OF THE BLOOD OF FOWLS

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In earlier studies on metabolic differences between the male- and female-producing eggs (yolks) of pigeons, it is shown by one of us (1, 2, 3, and much unpublished data), that maleness and femaleness in this earliest stage of the organism differ quantitatively in their capacity to *store* material within themselves. The substances which are there elaborated and stored—to a degree unusal in most tissue cells—are chiefly fat and phosphatides. The question naturally arises as to the continuance of this original distinction between eggs of prospectively different sex-value into embryonic and adult stages. Do similar metabolic differences characterize the adults of the two sexes? Several studies have been undertaken in an effort to answer these questions. In the present paper we present the results of a first inquiry into the amount of fat and phosphorus in the blood, in relation to sex and sexual activity, in the common fowl.

At this point it may be stated that the following data demonstrate that the blood-plasma of female fowls—in both their laying and non-laying periods—contains more alcohol-ether-soluble substance, and more phosphorus, than does the plasma of the male; and that these substances are more abundant in sexually functioning females than in owls with inactive ovaries.

METHODS OF PREPARATION AND ANALYSIS

The blood was drawn from the jugular vein through a paraffined cannula into a paraffined beaker; the bird having been previously given an injection of 2 to 2.5 cc. of a 0.1 per cent hirudin solution (in NaCl +

oxalate solution). Only local anaesthesia by cocaine was used, and this was used sparingly. The collected blood was centrifuged 2300 revolutions per minute for ten minutes, in 50 cc. haematocrits; the volume of corpuscles was recorded, and the available plasma decanted into a weighing tube, and thence to a porcelain evaporating dish. The samples were then placed for 9–24 hours—according to size of sample—at 85° in a drying oven to make them brittle, and to reduce to a volume convenient for an extraction cup.[1]

The crushed sample was at once extracted for four hours with alcohol in a Koch extraction apparatus; then for one hour, with ether, after which the sample was removed, pulverized, and returned for a twelve hour extraction with alcohol. The phosphorus was determined by the Neumann-Pemberton method on both the alcohol-soluble and insoluble fractions of the plasma. The figures for phosphorus are given in terms of lecithin (phosphatides); this is done here partly because it facilitates a comparison with the phosphorus determinations on the several kinds of tissue being studied by us, in which the phosphorus is uniformly so expressed. The methods of analysis throughout are also the same as those used in the analysis of other tissues reported upon in this series of papers.

Two samples used for preliminary total phosphorus determinations, and referred to in the text only, are of unseparated plasma and corpuscles to which sodium oxalate was added in the collection of the sample.

The egg-laying history (for one or more years) of the birds used was known from their trap-nest records. The ovary of every female killed was also examined to make certain the distinction between the laying and non-laying fowls. In every case the findings in the ovary and the trap-nest records are in agreement.

PRESENTATION OF DATA

In table 1 are given the results of the analyses of the plasma of the birds of the three groups—males, non-laying females, and of females known to be producing and laying eggs. It will be seen that the amount of alcohol-soluble substance and phosphorus is lowest in the males, clearly higher in the non-laying females, and very much higher in the laying females. The relative amounts of phosphorus in the three groups is expressed by the figures, 100 : 115 : 205. The alcohol-soluble materials are similarly expressed by the figures 100 : 116 : 181. It is clear that the proportion of alcohol-soluble substance, and total phosphorus, in the plasma of these three groups, mark them off as natural groups. The numbers are not large, but the differences are pronounced and practically conclusive. Only one of the figures within any group over-laps any figure in either of the other groups. This is for the phosphorus content of the plasma of a male, and is still far below the amounts of phosphorus found in any of the actively functioning females.

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[1] Some oxidation of unsaturated fatty acids doubtless occurred during this period of drying.

Further evidence that the non-laying condition is accompanied by low fat and phosphorus content of blood, as compared with the active egg-laying condition is to be had from two sources: (a) The third analysis (April 11, table 1) in the section for laying fowls was of a sample obtained from the bird having by far the poorest egg-laying record of any of the five of the series. Her blood proved to be markedly poorer in fat and phosphorus than the others of the series, and yet clearly richer in these substances than any of the fowls which were laying no eggs whatever. (b) The results of two preliminary phosphorus determinations on two samples of blood (plasma + corpuscles) of non-laying females supply additional confirmations of this relation. The figures obtained were 6.50 per cent and 7.23 per cent phosphatids[2] (calculated as lecithin); since there are considerable amounts of both phosphorus[3] and alcohol-soluble substance in the corpuscles of birds, and since the latter make up only about one-third to one-fourth of the total volume of these blood samples it is clear that the results of these two determinations confirm those made on the plasma alone.

Sufficient additional data are included in table 1 to show that neither age (within the limits used), nor size, nor breed of bird, is responsible for either of the three observed levels at which fat and phosphorus exist in the blood-plasma. The amount of plasma used in each analysis, and the percentage of water found in each sample are also recorded.

Of considerable interest is the distribution of phosphorus between the alcohol-soluble and alcohol-insoluble fractions; and further the variation in this distribution in the three groups of birds. A complete

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[2] These percentages are not strictly accurate for total blood samples since correction has not been made for the amount of increase of total solids due to the [illegible] the sample.

[3] [illegible] gives the amount of phosphorus [illegible]

TABLE 1

Analyses of blood-plasma from males, non-laying, and laying female fowls

Males (roosters)

DATE	DESCRIPTION OF BIRD	WEIGHT	WEIGHT SAMPLE	WATER	ALCOHOL SOLUBLE	PHOSPHATIDES*	RATIO OF P. IN 3 SERIES
			grams				
April 11	Wh. Wyandotte (mong.) 2 years old..	2,282	111.90	95.23	15.57	5.29	100
April 21	Wh. Wyandotte (mong.) 2 years old..	2,410	88.38	94.63	15.32	8.62	
November 23	Mongrel...............	2,480	46.10			5.39[2]	
	Non-laying females						
December 2	Wh. Wyandotte 1½ years old..........	1,405	19.90	94.48	17.46	7.90	115
December 18	Houdan 1½ years old...	1,375	42.95	94.13	18.21	6.62	
December 18	Wyandotte-Houdan (mong.)? 3 years?....	2,015	37.80	93.34	17.93	7.75	
	Females laying eggs						
April 5	Game, 3–4 years old....	1,750	42.00	93.47	30.52	13.83	205
April 5	Plymouth Rock, 3 years old............	1,500	47.78	93.86	28.20	14.81	
April 11	Wh. Wyandotte (mong.) 1 year old†..	1,404	42.51	94.38†	21.99†	9.86†	
April 11	Wh. Wyandotte (mong.) 1 year old...	1,332	48.67	94.02	33.63	14.39	
April 21	Wyandotte-Houdan (mong.) 11 months old	1,380	28.69	94.17	25.07	12.84	

* Phosphorus calculated as lecithin.

† This bird laid only two eggs during the nine days preceding the taking of the sample, the poorest laying record of any female of this series.

view of this situation is to be had by reference to table 2. The alcohol-soluble fraction contains 2.5 to 4.5 times more phosphorus than does the insoluble fraction. But it will be noted that the greatest disproportion of the two is found in the males; least in the actively laying females; and that here again the non-laying females form the intermediate group. The relation within the three groups of the distribution of phosphorus in the two fractions of the analysis, is a further evi-

TABLE 2

The distribution of phosphorus in the alcohol-soluble and insoluble fractions—(analyses of table 1)*

ANALYSIS	MALES		NON-LAYING FEMALES			LAYING FEMALES				
	April 11	April 21	December 2	December 18	December 18	April 5	April 5	April 11	April 11	April 21
Alcohol soluble....	18.87	21.84	20.80	16.71	17.61	26.10	25.92	21.38	21.80	20.00
Alcohol insoluble..	2.78	6.23	5.17	4.36	5.61	8.44	10.45	6.61	10.63	10.44
Ratio insoluble to soluble..........	1 : 4.5		1 : 3.6			1 : 2.5				

* Calculated as lecithin.

dence that the three groups of birds, are natural groups in reference to the fat and phosphorus content of the blood-plasma.

GENERAL CONSIDERATIONS

Apparently there are but few published observations bearing on the relative amounts of fat and phosphorus in the blood of males and females; we know of none which deal solely with the blood-plasma of a vertebrate. A still smaller number of published data deals with the changes in the quantity of these substances in the blood or plasma of females in relation to periods of ovarian rest and activity.[4]

Gorup-Benanez (4) makes the meager but interesting tabular statement that the blood of males contain *less* fat, that of females *more*.[5] Miescher (5) noted that at the time of ripening of eggs in the ovaries of the salmon the blood-serum contained "erhebliche Mengen" of lecithin and fatty acids. Heim (6) showed that in a number of species of Decapod Crustacea the blood of the females acquires a brilliant yellow lipochrome (alcohol-soluble) pigment at the time of the ripening of the ovaries.

The work of Geoffrey Smith upon the blood of the crab (Carcinus maenas) is, however, of particular interest in connection with the present study. Smith made adequate quantitative determinations of the total ether-soluble contents of the blood of males which were maturing their eggs. This amount was found to be much greater than that similarly obtained from the blood of the males approaching moult; and also very much greater than the colorless combined bloods of males and females. Unfortunately, perhaps, for our purpose, the blood of males and females were not compared at the period of their lowest fat content.

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[4] The changes in quantity of lipoids of the blood in disease, and the normal and experimentally induced fluctuations in the fat content of the blood, do not really require consideration in connection with the current topic. Some of these will, however, be considered in other of these studies.

[5] Since this paper was written a paper by Goettler and Baker (Journ. Biol. Chem., xxv, June, 1916) bearing on this subject has been published. The fat and cholesterol of male and female human (normal) blood was determined, be-

Smith's work has additional merit because of its elucidation of the relation of the blood changes to other carefully studied somatic changes which occur under the so-called "parasitic castration" of these forms by *Sacculina*—a condition in which the males tend to take on female secondary sexual characters, young females tend to hasten the assumption of such adult female characteristics, and the blood of both sexes becomes much richer in fat and lipochrome pigment. Additional interest pertains to these studies because, on the basis of the results, Smith has formulated a theory of sex to fit all the facts as observed in Carcinus and related crabs.

It will thus be seen that Smith has clearly recognized and studied a connection between certain aspects of sex and fat-content of the blood. Though we cannot here adequately consider the theory advanced by Smith it should be noted, that he has not assumed or sought

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sides 26 other substances. The authors do not call attention to the sex differences of the fat estimations, but if we summarize and arrange their data according to sex it will be found to be as follows.

SEX	FAT			CHOLESTEROL			FAT + CHOLESTEROL
	Max.	Min.	Av.	Max.	Min.	Av.	
Man	128.0	66.4	102.1	58	17	42	(18) 141.4
Woman	320.0	145.0	199.6	44	19	29	(5) 226.0

The figures indicate milligrams per 100 cc. of blood. In addition to the above a *diabetic* male gave a fat figure of 158.8. The increase in cholesterol does not seem to keep pace with the increase in fat. It is clear that the figures of Goettler and Baker indicate a higher fat content of the blood of the human female. In connection with the division of females made in the present paper it is interesting to note that the two females whose ages were 30 and 35 years give an average of 253.5 for fat in blood; while three women of 48 to 59 years show an average of only 163.7. The *functional condition* of the *ovary* would seem to be associated with the highest fat values in the blood, whether that ovary utilizes much fat (fowl) or little (woman).

for *initial* differences in fat metabolism, but holds that the parasitic Sacculina—by depriving the blood of the male host of circulating fat as does the ovary of the female—stimulates a continual production of yolk (= "sexual formative substance") which thus accumulates in the blood, and whose presence in the blood causes the parasitized (castrated) male to assume the characters of the female. Some features of Smith's theory would seem to possess obvious advantages over some current conceptions. It remains to point out, however, that the results obtained from the study of the crab's blood— and as we believe on the general topic of 'parasitic castration' in crabs—are in entire agreement with the theory of sex which has been stated by Riddle (1, 2, 3) and that, in so far as the blood analyses contrast the blood of males and females, the results are similar to those described in this paper. No phosphorus values were obtained from the crab's blood; and the blood of the normal "inactive" male and female was not compared.

When one begins from the point of vantage stated at the outset of this paper—the demonstated initial difference in storage metabolism (particularly of fat and lecithin) of male- and female-producing ova of the pigeon—it is clear that ever new and subsidiary hypotheses are unnecessary for the general understanding of many hitherto puzzling sex phenomena. A higher fat content of the blood of woman, female crab, and hen, than of man, male crab, and cock, is, on Riddle's view, the expected result of a mere continuance or persistence of the observed relative powers of the eggs.

The eggs which prospectively will produce organisms of the two sexes are themselves already differentiated in respect to their fat and phosphorus metabolism[1] in precisely the same direction as are the plasmas of adult males and females. The female-producing egg is more plentifully supplied with fat and phosphorus than is the male producing egg (Riddle). The metabolic levels of the eggs accord with that difference. The blood and plasma thus far investigated indicate that the metabolic levels of the two kinds of eggs are continued from the germ through the embryogeny into the adult.

SUMMARY

A difference [illegible] in the fat and phosphorus metabolism of male-

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[1] The [illegible] in the plasma of males [illegible] actively laying females [illegible] differentiated.

and female-producing ova in the pigeon (Riddle) invites a comparison of the quantities of these substances present in the adults of the two sexes.

The blood-plasma of female fowls is found to be richer in alcohol-soluble substance and phosphorus than is the plasma of the male.

The blood-plasma of the sexually functioning female fowl contains more alcohol-soluble substance and more phosphorus than does the plasma of fowls with temporarily inactive ovary.

The male, the non-laying female and the actively laying female fowl are three natural groups of fowls when these are considered from the standpoint of the fat and phosphorus content of the blood-plasma. The relative distribution of phosphorus in the alcohol-soluble and alcohol-insoluble fractions of the blood-plasma is also different for these three groups of fowls.

The differences here observed and measured are quantitive not qualitative in kind.

These results on the fowl, together with the few data available on other forms, lend support to the view advanced by Riddle that the initial metabolic differences between eggs of different sex value persist in the organisms developed from the eggs of the two grades.

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THE RELATION OF ACID TO GASTRIC DISCHARGE AND DUODENAL REGURGITATION IN THE DOG

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From a review of physiological literature one receives the impression that the presence of an acid reaction in the stomach is essential to and hastens gastric discharge. This is not entirely concordant with clinical observations. In cases of so-called hyperacidity a retarded emptying of the stomach with resulting atony and dilatation is often found. Also in cases of achylia gastrica, where there is practically no free acid found in the stomach, the normal rate of discharge is often unimpaired. It therefore seems probable that the presence of an acid reaction is not the only essential factor in the control of gastric discharge.

Hirsh (1) has reported that solutions of inorganic acids leave the stomach slowly and inferred that this slow exit was due to the stimulating effect of the acid on the mucosa of the duodenum. Cannon (2) in his fluoroscopic work on cats, fed mashed potato and bismuth, and observed that hastening the appearance of an acid reaction hastened the initial gastric discharge. Hedblom and Cannon (3) fed acidified mashed potato and bismuth to their animals and by fluoroscopic observations estimated that the rate of discharge was increased over the normal rate up to an acidity of 0.25 per cent, acidity above 0.25 per cent reduced the rate of discharge to or below the normal. Heidenhain (4), Pawlow (5), Sommerfeld (6), Carlson (7), and others have demonstrated an acidity of 0.4 to 0.5 per cent in the pure gastric juice of animals and man. Boldyreff (8), has presented evidence that this high acidity is neutralized to an optimum acidity of 0.15 to 0.2 per cent by regurgitation of duodenal juices of which the pancreatic juice is most important. Through a gastric fistula in dogs, Boldyreff injected acidified fluids into the stomach and found that the acidity was rapidly lowered to 0.15 to 0.2 per cent and the rate of discharge was increased with the reductions of the acidity. After ligating the pancreatic ducts he found that the reduction of the acidity and the rate of discharge were greatly retarded. Recently Spencer, Meyer, Rehfuss and Hawk (9), have demonstrated the presence of pancreatic trypsin in the gastric contents, the amount present being greater with low acidity than with high acidity. In the same article these investigators noted that sodium bicarbonate in 1 per cent solutions left the stomach rapidly while 5 per cent left slowly. By means of the X-ray Tabora (10) studied gastric peristalsis and motility in normal individuals. He used bismuth alone and combined with hydrochloric acid. When acid was used the peristalsis was deeper and more energetic but the emptying time was 50 per cent longer. Carlson (11) found that the rhythms of the empty stomach appear whether the mucosa is alkaline or acid, but are inhibited by strong acidity or alkalinity. The inhibition was greater with 0.5 per cent hydrochloric acid than with a similar concentration of sodium carbonate. Clinically considerable variation in the relation of acidity to discharge is also found in observations made upon patients with so-called hyperacidity. Osler (12) finds that most cases of hyperacidity show a normal or increased motility although in 30 per cent of his series there was a coincident atony of the stomach with some retention. Strümpell (13) usually finds the stomach empty within three to four hours after a test meal, which he attributes to increased motility and an increased digestive power of the gastric juice. Reed (14) maintains that hyperacidity and some degree of gastric hyperesthesia which frequently is coincident, are the chief causes of the common motor disturbances such as spasm of the cardia and of the pylorus with long delayed emptying of the stomach. Kemp (15) states that motility is unimpaired in cases of hyperacidity, unless complicated with atony and insufficiency which it often produces. Krehl (15) considers hyperacid secretions with resulting pylorospasm as an etiological factor in gastric dilatation.

From the above noted somewhat conflicting experimental and clinical observations it is evident that the relation of gastric acidity to motility, discharge, and duodenal regurgitation is still sufficiently problematic to justify further investigation under varying conditions. The conditions imposed in this research were first: a normal fasting animal stomach, second, fluid injections of constant temperature and quantity but of varying acidities; third, abolishment of inhibiting sympathetic reflexes by pithing the spinal cord.

TECHNIQUE

Dogs were used throughout the experiments. The animals were unfed for forty-eight hours to secure total absence of food from the stomach. This however was not obtained in every case, as noted below, owing to the indigestible nature of some of the material previously ingested. Light ether anaesthesia was maintained by the tracheal cannula-ether bottle method. Through a laminectomy incision the spinal cord was destroyed from the sixth to the tenth dorsal segments. This destruction of the cord severed the splanchnics from their connection with the central nervous system and thus eliminated inhibitory reflexes. Cannon (17) determined that section of the splanchnics in cats resulted in no change of the normal movements of any part of the alimentary canal. Carlson (18) found that section of the splanchnics in dogs increased the gastric tonus and augmented the gastric hunger contractions. It is therefore evident that destruction of the inhibitory mechanism does not diminish gastrointestinal activity. Fluids were introduced and withdrawn from the stomach by means of a glass tube inserted through an esophageal fistula in the cervical region. The fistula was made to avoid laryngeal reflexes and prevent swallowing of saliva. The portion of the tube used for insertion into the stomach was bent at an angle of 10 degrees 1 inch from its termination and provided with a rubber extension. The protruding portion was also bent in the same plane as an index to the direction assumed by the bent portion of the inserted end. The protruding end was connected with a funnel for introduction of fluids. When aspirating the stomach contents a suction bulb was substituted for the funnel. By placing the animal on its left side and directing the bend in the tube toward the animal's left, the most dependent portion of the fundus of the stomach could be reached and completely aspirated.

At the beginning of each experiment, after removing the fluid contents of the stomach, our aspirating technique was tested and the stomach lavaged by the introduction and immediate withdrawal of 200 cc. of water. If 198 to 200 cc. were recovered the method was considered efficient, 2 cc. being allowed for immediate discharge through the pylorus. If less than 198 cc. were recovered the tube was readjusted and the test repeated until satisfactory results were obtained. It was seldom found necessary to repeat the test. By carefully measuring the amount of fluid introduced into the stomach and the amount withdrawn, the amount of discharge or of duodenal regurgitation may be determined. The question immediately arises however: What effect has the introduction of water and various acid solutions on the rate of *gastric secretion?* This secretion, if considerable, would be a disturbing factor in the results. Pawlow (19) found that in dogs gastric secretion is slightly stimulated by the introduction of 500 cc. of water into the stomach. The introduction of 100 to 150 cc. of water produced no secretion in 50 per cent of his cases, the secretion in the case of 500 cc. of water being due to the long continued contact of the water with the gastric mucosa. Solutions of meat ash, chloride and hydrochloric acid produced exactly the same effect as water alone. It was therefore considered probable that the amount of secretion produced when 200 cc. of water or hydrochloric acid solutions are introduced is small and not a disturbing factor in the comparative values of discharge or duodenal regurgitation. In order to determine positively that secretion is not a disturbing factor under the conditions of this investigation, a separate series of experiments was made upon six animals using the same technique but with the pylorus ligated. The results of these experiments usually showed no secretion, and when any occurred it was so small in amount as to be negligible. This series will be extended and reported in detail. When more fluid was recovered than was introduced the increase was attributed to duodenal regurgitation as gastric secretion or hemorrhage could not be considered sufficient to produce the results.

In the above noted technique the stomach and intestines were not traumatized. The abdomen was not opened until the end of the experiment when an autopsy was made and the gastric contents noted as a further check upon the aspirating efficiency. Previous to the adoption of this technique two other methods were tested. First the rate of gastric discharge was determined by recovering the discharged fluid from the duodenum through a fistula. Second a tube was introduced into the stomach through an incision in the stomach wall and fluids were introduced and withdrawn the discharge being noted by difference. Both of these methods were discarded because of local inhibitions produced.

Data were obtained from two series of experiments. In the first fourteen animals were used. Solutions of acidity varying from 0 to 0.5 per cent hydrochloric acid were introduced into the stomach.

The quantity used was uniformly 200 cc. and the temperature 37°C. Water was first injected. The acidity was increased 0.1 per cent with each injection until 0.5 per cent hydrochloric acid was reached. Each injection was aspirated after an interval of thirty minutes. The results from two of the animals of this series were discarded as unreliable. In the first case aggravated retching produced regurgitation with loss of considerable fluid thus destroying the accuracy of the measurements. In the second case 25 cc. of fluid and some solid material were found in the stomach at autopsy.

In the second series four animals were used. The time interval was shortened to twenty minutes and the acidity of the solutions was increased 0.2 per cent with each injection until 1 per cent hydrochloric acid was reached.

The post mortem examination of the stomach contents as a check upon the aspirating technique was usually gratifying, In six animals of the first series the stomach was empty, in two there was a small amount of solid material, such as bone and remnants of potato but no fluid; in one there was 25 grams of solid material and 4 cc. of fluid. In the remaining three animals of this series a small amount of thick discolored mucus was found. In the second series two stomachs were empty, one contained some mucus and one some solid material but no fluid.

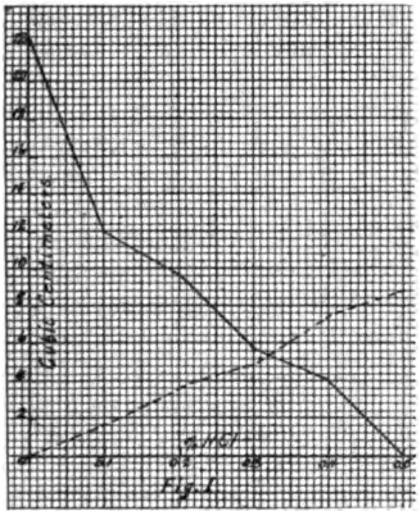
Chart I

DOG NO.	H_2O		HCl–0.1%		HCl–0.2%		HCl–0.3%		HCl–0.4%		HCl–0.5%	
	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.
1	20	0	25	0	0	10*	5	0	0	0	0	0
2	27	0	27	0	0	18*	0	20	20	0	0	25
3	5	0	0	0	25	0	5	0	0	18*	0	0
4	50	0	25	0	35	0	15	0	0	5*	0	25
5	20	0	5	0	10	0	10	0	0	15*	0	5
6	5	0	0	0	0	15*	0	0	0	30	0	10
7	55	0	50	0	5	0	0	7*	0	3	0	0
8	55	0	0	20*	10	0	10	0	20	0	0	20
9	5	0	0	0	7	0	5	0	0	0	0	10*
10	10	0	0	0	20	0	0	30*	0	0	0	10
11	10	0	5	0	0	0	10	0	10	0	0	5*
12	10	0	5	0	0	0	10	0	0	10*	0	5
Average.....	$22\frac{1}{4}$	0	$11\frac{10}{12}$	$1\frac{8}{12}$	$9\frac{1}{3}$	$3\frac{7}{12}$	$5\frac{5}{6}$	$4\frac{3}{4}$	$4\frac{1}{6}$	$7\frac{1}{2}$	0	$8\frac{3}{4}$

D, discharge from stomach; R, regurgitation into stomach; *, first appearance of regurgitation.

RESULTS

The results of the first series as tabulated in Chart I show a general uniformity although certain deviations are noted. In eight of the twelve animals the greatest discharge was obtained with water. The average discharge with water was 22.2 cc., which is less than that obtained by Moritz (20) from duodenal fistulae. The average discharge for the various dilutions decreased with increase of acidity (fig. 1). These findings are in accord with Hirsch's (1) finding that inorganic acids leave the stomach slowly.



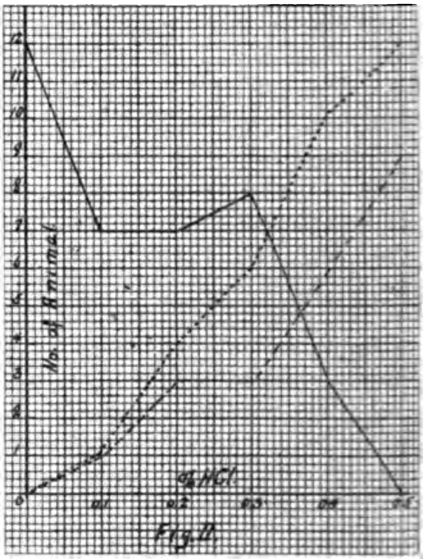


Fig. 1. Based on 12 experiments, ——— average discharge; - - - - - average regurgitation.

Fig. 2. Based on 12 experiments. ——— Number of animals showing discharge. - - - - - Number of animals showing regurgitation. ...... Number of animals having shown regurgitation at some previous stage of the experiment.

Referring to Chart I the following individual deviations are noted. Dogs 1, 3, 9 and 10 have slightly greater discharge with acid solutions than with water. Dogs 2, 3, 4, and 8 have at some point a greater discharge with acid solutions than with some preceding lesser acidity but not greater than with water.

In addition to the progressive diminution in the amount of discharge there is an increase in the average amount and in the frequency of regurgitation with increase in the acidity (figs. 1 and 2). In every case regurgitation occurred at or before 0.5 per cent acid was reached. Hicks and Fisher (21) recently reported regurgitation in 38 per cent of 56 trials on dogs when 0.5 per cent hydrochloric acid was introduced into the stomach. After section of the splanchnics they obtained regurgitation in all of nine trials. Change in color was considered sufficient evidence of regurgitation by these investigators after they found that one drop of bile in 100 cc. of water produced a bright straw color. In our series of experiments regurgitation was evidenced by the increase of the fluid content of the stomach. The appearance of bile coloring in the aspirated fluid was occasionally noted but if constantly present was usually obscured by slight hemorrhage which occurred between 0.3 and 0.9 per cent acidity. A control color test with blood and hydrochloric acid solutions showed that from 1 to 3 cubic centimeters of blood in the aspirated contents would produce the color change and hence hemorrhage was not sufficient to account for the increase of the gastric contents. The mucosa at autopsy invariably showed excoriated areas more numerous in the fundus than in the antrum.

In every case some discharge was obtained with water. Occasionally when using acidified fluid the amount aspirated was the same as that introduced thus giving us no evidence of discharge or regurgitation. The number of animals showing discharge decreased with increase of acidity up to 0.5 per cent acid, where a discharge was never obtained (fig. 2).

Chart II

DOG NO.	H_2O		HCl–0.2%		HCl–0.4%		HCl–0.6%		HCl–0.8%		HCl–1.0%	
	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.	D.	R.
1	10	0	0	0	5	0	0	5*	0	10	0	20
2	0	0	0	0	0	0	0	5*	0	15	0	50
3	60	0	0	20*	0	5	0	30	0	5	0	5
4	8	0	0	0	0	0	0	0	0	0	0	10*
Average.....	19½	0	0	5	1¼	1¼	0	12¼	0	7½	0	21½

D, discharge from stomach; R, regurgitation into stomach; *, first appearance of regurgitation.

The results of the second series (Chart II) with higher percentages of acid in general corroborate the findings of the first series. In only one case did any discharge occur except with water. Dog No. 1 shows 5 cc. discharge with 0.4 per cent acid, but this is 6 cc. less than that obtained with water.

DISCUSSION

The most prominent feature of the results, as tabulated above, is the progressive diminution in the rapidity of discharge with increase in the acidity. This does not concur with the findings of Hedblom and Cannon (3) in which the optimum acidity for maximum discharge was found to be 0.25 per cent. However Hedblom and Cannon's work was done on cats fed mashed potato and bismuth. At autopsy of their animals they found the material fed was separated into masses in the intestines (22). Possibly the difference between their results and those herein reported may be explained by the fact that only the surface of the semi-fluid material passing through the pylorus was presented to the duodenal mucosa. In this diffusion would be retarded and the acidity of the surface could be quickly neutralized by the alkaline duodenal juices without exciting inhibition of discharge or regurgitation. This process could continue as the peristaltic action of the duodenum brought new surfaces of the material in apposition with the mucosa. But in the case of acidified fluid, the whole acid content is in effect at once presented to the mucosa and the neutralizing power of the duodenal juices is exhausted before the volume of fluid admitted is neutralized below the point sufficient to produce inhibition of discharge or regurgitation.

Boldyreff (8) finds the optimum acidity to be 0.15 to 0.2 per cent. He believes that the constant acidity of 0.4 to 0.5 per cent of the gastric juice is reduced by influx of duodenal juices. This view is supported by the presence of trypsin in the gastric contents as demonstrated by Spencer, Meyer, Rehfuss and Hawk (9). The constant occurrence of regurgitation as noted in our experiments may be taken as further evidence in support of Boldyreff's theory. This regurgitation always occurred at 0.5 per cent acid or before 0.5 per cent acid was reached and the average amount of regurgitation as evidenced by the quantitative estimation was directly proportional to the acidity of the fluid in the stomach.

Throughout these experiments the presence of acid in the stomach in general has been observed to retard rather than hasten gastric discharge. As noted above Spencer, Meyer, Rehfuss and Hawk (9) observed that 1 per cent sodium bicarbonate leaves the stomach rapidly, while 5 per cent leaves slowly. In the light of their observations and the results of this series of experiments it appears that the acidity of fluid gastric contents has no function in producing or hastening gastric discharge, but the discharge seems to be controlled by the irritability or non-irritability of gastric contents to the duodenal mucosa.

The optimum acidity as reported by different investigators and as observed in these experiments seems to vary with the physical condition of the material used and is lower for the more fluid content. The optimum acidity seems to be that which can be quickly neutralized or diluted to a non-irritating point without exciting inhibition of discharge or regurgitation.

SUMMARY

1. Water is discharged from the fasting stomach of anesthetized pithed dogs more rapidly than any percentage of acid.
2. The rate of discharge is decreased with increase of acidity.
3. Duodenal regurgitation, as evidenced by increase in the contents of the stomach, often occurred at 0.2 per cent acidity and in nearly all trials occurred at or before 0.3 per cent acidity was reached.
4. Increase in acidity increases the frequency and amount of regurgitation from the duodenum.

I wish to express my gratitude to Dr. R. G. Hoskins and Mr. R. E. Lee Gunning for their many valuable suggestions, and assistance throughout the work.

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STUDIES ON BIOLUMINESCENCE[1]

II. On the Presence of Luciferin in Luminous Bacteria

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In a recent issue of Science (1) I pointed out that Dubois (2) had demonstrated in 1885 the existence of two substances which are concerned in the light production of the West Indian Cucullo, *Pyrophorus noctilucans*, and also of the boring mollusc, *Pholas dactylus*, one, thermostabile, called luciferin; and another thermolabile, called luciferase and having all the properties of an enzyme. Dubois demonstrated this by filling two test tubes with an extract (luminous) of the luminous organs, then boiling one tube, which destroys the luciferase but not the luciferin, and allowing the other tube to stand until the light disappears, i.e., until all the luciferin is completely oxidized in the presence of oxygen. If the tube contents (both dark) are mixed, light appears. Luciferin and luciferase have now been brought together in presence of oxygen and the light lasts until the remaining luciferin is completely oxidized. I have been able to verify the above for *Pyrophorus* and to show that the common American fire-flies contain luciferin and luciferase; also to demonstrate that luciferin from one genus of fire-fly (*Photinus*) will act with luciferase from another genus (*Photuris*) and vice versa; also that fire-fly (*Lampyridae*) luciferase will act with *Pyrophorus* (*Elateridae*) luciferin and vice versa. Non-luminous parts of fire-flies contain no luciferase, however, nor do non-luminous insects or extracts of earth-worms, slugs or pill bugs (3).

Oxygen is essential for light production by luciferin and luciferase, but if oxygen is absent the luciferase is able to change the luciferin in some way. This may be shown by allowing an oxygen-free aqueous mixture of luciferin and luciferase to stand. If oxygen is admitted in five minutes we obtain light but if we wait for one hour before admitting oxygen, no light is produced (4). Luciferin alone or luciferase alone in solution are stable for over six hours.

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[1] The first paper of this series entitled, Studies on Light Production by Luminous Bacteria appeared in this Journal, 1915, xxxvii, 230. The third paper follows the present one. Papers upon the chemistry of luminescence in the fire-fly and in luminous marine forms will follow.

Luminous bacteria contain luciferin also but in very small quantities as we might expect from the intensity of the light which is far less brilliant than the fire-fly although produced continuously. I have been wholly unable to demonstrate it by a repetition of Dubois' experiment, due in part I think to the fact that in bacteria the luciferase is an endoenzyme which cannot be extracted by the ordinary biochemical methods. If we use the luciferase of the fire-fly we can obtain light from a bacterial luciferin prepared in the following way: by adding absolute alcohol to a dense mass of the bacteria, then removing the alcohol by centrifuging and quickly drying by evaporation in vacuo. The resultant powder gives no light with water but does phosphoresce very faintly with a solution of fire-fly luciferase. Similar material prepared with acetone instead of alcohol gave no light with fire-fly luciferase, nor did bacteria heated from one-half to two minutes to 100°C. Thinking that bacterial luciferin as well as the luciferase might be destroyed below 100°, I tried heating to lower temperatures, 90°, 80°, 70°, 60° and 50° for two minutes, but in no case was light produced when fire-fly luciferase was added to these bacteria. Probably the amount of luciferin in luminous bacteria is so small that it is burned up before the temperature rises sufficiently to destroy the luciferase, whereas, addition of alcohol instantly prevents the action of the luciferase. In order to obtain a bacterial powder which will give light with fire-fly luciferase it is necessary to remove the alcohol and dry as quickly as possible; otherwise the luciferin is also destroyed.

Addition of ether to a mass of luminous bacteria causes the light to disappear rapidly and I thought that the luciferase might be destroyed and not the luciferin, but bacteria so treated, after the ether had been evaporated by a current of air, gave no light with fire-fly luciferase. Negative results were obtained also with an alkaline ($\frac{M}{15}$ KOH) extract of luminous bacteria neutralized with HCl. A precipitate of nucleoprotein is produced on neutralization. Apparently the bacterial luciferin is formed in such small quantities from its precursor or is so unstable that all methods of obtaining it have failed with the exception of the rapid precipitation by alcohol.

That bacterial luciferin, like fire-fly luciferin, is changed and disappears on standing in contact with *oxygen-free* water, was demonstrated by my earlier attempts to extract a photogenic substance with oxygen-free solvents (5).

It may be pointed out in passing that the experiments with bacterial luciferin were made with an unfiltered suspension of the bacteria since they are small enough to pass filter paper. Fire-fly luciferin may be obtained in a perfectly clear filtrate free of cells and cell fragments.

On the other hand, I have found it impossible to obtain a bacterial luciferase. Neither bacterial luciferin nor fire-fly luciferin will react with any sort of bacterial preparation which might be supposed to contain luciferase. I have tried extraction with water and chloroform water of both the fresh bacteria and those rapidly dried and then ground with sand. The methods of growing, collecting and drying the bacteria are described in my earlier paper (6). It is not surprising that this is so, for many enzymes are found in bacteria in a condition which defies extraction except by means of the Büchner press and it is difficult to obtain luminous bacteria in sufficient quantity for this method. I feel certain that the oxidases are contained in these bacteria in this endoenzyme condition. All my efforts to obtain an oxidase in solution which will oxidize the common oxidase reagents; guaiac a-napthol, para-phenylen-diamine, a mixture of a-napthol and para-phenylen-diamine, phenolphthalin and pyrogallol have failed, even though hydrogen peroxide is added.

Similar results have been obtained by other workers. Gessard (7) showed that a melanogenic variety of *Bacterium pyocyaneum* would turn tyrosin brown but he was unable to separate a solution of tyrosinase from the bacteria. Lehman and Sano (8) found also that *Actinomyces chromogenes*, *Bacterium putidum* and *B. phosphorescens* would oxidize tyrosin in the culture medium but *Vibrio indicus*, which phosphoresces strongly, *Sarcino lutea*, *B. typhi*, *B. coli*, and many others would not. They found in addition that a substance oxidizing aloin and giving a very weak guaiac reaction could be extracted from *Actinomyces chromogenes*, *B. putidum and B. phosphorescens* by a mixture of two parts glycerine to one of water, but no tyrosin reaction could be obtained with this extract. Here also the tyrosinase is apparently in an endoenzyme condition.

Although luciferase is no doubt an oxidase it is not like the oxidases of plant juices, of potato or turnip juice for example. Fire-fly or bacterial luciferin and turnip juice or potato juice will give no light when mixed even upon the addition of H_2O_2. Fire-fly luciferase is also very readily destroyed by chloroform water while the oxidase of turnip juice can be preserved indefinitely by chloroform. Fire-fly luciferin is not destroyed by chloroform but a solution of luciferin preserved under chloroform loses its power to phosphoresce in the course of ten to twenty days. In many ways the luciferin of the fire-fly is much the more stable substance. A complete account of its properties will be found in a subsequent paper of this series.

SUMMARY

1. Attention is called to the discovery of R. Dubois in 1885–1886 of the presence of a thermostabile, oxidizable substance, *luciferin*, and a thermolabile enzyme-like substance, *luciferase*, in the luminous organs of the mollusc, *Pholas dactylus* and the beetle, *Pyrophorus noctilucans*.
2. Similar substances occur in the American fire-flies and can be obtained in solution filtered through filter paper.
3. Luciferin from *Photinus*, *Photuris*, or *Pyrophorus* will give light with luciferase from any of the above three genera or vice versa.
4. Non-luminous parts of fire-flies or extracts of many other non-luminous insects, slugs, earth-worms, or pill bugs give no light with luciferin.
5. Luciferin in a very impure state can be obtained by precipitating luminous bacteria with alcohol and will give light with fire-fly luciferase.
6. Luciferase cannot be obtained from luminous bacteria because it is probably present as an endoenzyme.
7. Oxidases for guaiac, a-napthol, para-phenylen-diamine, phenol, phenolphthalin, pyrogallol, or indo-phenol formers, if present, are also in an endoenzyme condition.
8. In presence of oxygen, luciferase decomposes luciferin with light production; in absence of oxygen the luciferin is also decomposed but without light production.
9. Luciferase (fire-fly) is quickly destroyed by ether and chloroform, in this respect differing from the vegetable oxidases. Luciferin (fire-fly) is not readily destroyed by ether or chloroform.
10. Luciferin of bacteria or the fire-fly will give no light with the oxidases of potato juice either with or without addition of hydrogen peroxide.

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STUDIES ON BIOLUMINESCENCE

III. On the Production of Light by Certain Substances in the Presence of Oxidases

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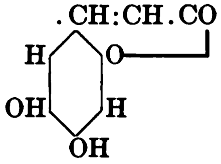
Since the researches of Radziszewski in 1877 (1) and 1880 (2) on lophin and various oils, alcohols and aldehydes we have come to recognize an ever increasing number of substances which will luminesce under appropriate conditions at temperatures approaching those compatible with life. Lophin (triphenylglyoxaline) gives an especially good light with alcoholic potash at 60°C., as do many essential oils. Trautz (3) has added additional substances to the list of chemiluminescent reactions, as has also R. Dubois (4), who discovered the luminescence of aesculin, a glucoside obtained from horse chestnut bark and chemically a combination of glucose and aesculetin.



These substances all require fairly strong alkali, although I have a sample of Bergamot Oil which gives a beautiful light when mixed with n/10 NaOH at 20°C. and a faint light at 5°C. At 20°C. a faint light appears with n/80 NaOH. On warming the light is brighter. n/80 NaOH is, however, incompatible with life. Lophin and aesculin will not luminesce except with alcoholic NaOH or KOH.

Ville and Derrien (5) made a step forward when they showed that lophin would luminesce with blood and H_2O_2 and Dubois (6) has recently discovered that aesculin also will give light with blood and H_2O_2. The blood contains an oxygen carrier, haemoglobin, which accelerates the oxidation of the lophin and aesculin. I can confirm the statement of Dubois, and of Ville and Derrien and find also, contrary to Dubois, that aesculin will give light with $K_2Mn_2O_8$ and H_2O_2. It is best to keep the temperature about 60°C. I found also that aesculin would give light with $FeCl_3 + H_2O_2$ but not with $FeCl_3$, or $K_2Mn_2O_8$ or H_2O_2 alone, nor with plant extracts rich in oxidases (turnip, potato and horse radish root) either with or without H_2O_2, and that blood extract still retains its power to produce light with lophin after boiling. Blood will oxidize guaiac after boiling and upon addition of H_2O_2. Oxonized turpentine can take the place of H_2O_2 in oxidation of aesculin.

It is of interest to note the similarity between the oxidation with light production of aesculin or lophin by means of the blood oxidases, and the oxidation of luciferin by luciferase, the substances found in luminous animals. Except that luciferase is instantly destroyed by boiling and is active at very low temperatures the parallel is indeed striking.

I have recently discovered (7) a luminescent reaction which mimics in all details the process occurring in luminous organisms and shows us the essential nature of that process. *The oxidation of a mixture of pyrogallol +* H_2O_2 *by the vegetable oxidases occurs with the production of light.* The reaction is highly interesting and remarkable for the following reasons. Perceptible light is produced with the concentration of pyrogallol m/32000 i.e., 1 part in 254,000 parts of solution; a faint light is produced at 0°C. and a bright light at 10°C.; KCN inhibits the reaction in m/2000 concentration; boiling destroys the oxidase just as it does luciferase.

The pyrogallol + H_2O_2 corresponds to luciferin and the vegetable oxidase to luciferase. The light produced is yellowish white and lasts two to four minutes with an intensity about equal to that of a suspension of luminous bacteria. The pyrogallol is converted into colored compounds by means of the oxygen of H_2O_2 which disappears in the process. As we shall see later, the oxidase is also used up in the reaction so that it does not function as a true enzyme. Whether luciferase is used up in oxidizing luciferin is unknown at present but I expect to present data on this subject in a subsequent paper. Hydrogen peroxide is necessary for light production even when potato juice is used as the oxidase solution, i.e., even when a juice containing an oxidase which will oxidize guaiac tincture directly (without addition of H_2O_2) is used. It is then strictly a peroxidase which is responsible.[1] Blood also gives the reaction. Catalase takes no part and may be destroyed by heating to 60°.

In studying this luminescent reaction more closely, I have used mostly pure potato juice (i.e., the pressed, unfiltered extract of grated potatoes, strained through cheesecloth and decanted from the starch grains) or a 1 per cent ox blood extract (i.e., 1 gram of dried defibrinated ox blood to 99 parts water) added to an equal volume of a half and half mixture of m/100 pyrogallol and 3 per cent H_2O_2 (Merck's Blue Label). The experiments will be given in the form of tables or data under the following heads:

1. Concentration of the three substances necessary for light production.
2. Substances taking the place of blood or potato oxidase, pyrogallol and H_2O_2.
3. Effect of temperature on the reaction.
4. Effect of KCN, NaOH and HCL.
5. Effect of fat solvent anaesthetics.
6. Is the oxidase a catalyzer?

1. Concentration of the three substances necessary for light production. (Tables 1, 2 and 3).

In general the more concentrated the blood or potato juice the brighter the light, and the more concentrated the pyrogallol (to a limit) the brighter the light, and the longer it lasts. Too strong pyrogallol precipitates the blood extract and potato juice, the former in weaker concentration than the latter (see table 1). In about m/8000 concentration the pyrogallol gives a bright light with potato juice and increasing the concentration above this does not increase perceptibly the brightness.

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[1] The difference between potato juice, which oxidizes guaiac without addition of H_2O_2, and turnip juice, which requires the addition of H_2O_2, appears to lie in the fact that the potato juice contains a substance which oxidizes spontaneously in presence of oxygen to a peroxide, while the turnip juice does not. Consequently, we must add a peroxide to the turnip juice. This spontaneously oxidizable substance was called by Bach and Chodat oxygenase. Both juices contain peroxidase. The "direct oxidases" (of potato) consist, then, of peroxide (oxygenase) + peroxidase, while the "indirect oxidases" (of turnip) are peroxidase alone. In this paper oxidase is used as a general name for an oxidizing enzyme and peroxidase for an enzyme transferring oxygen from a peroxide to an oxidizable substance. See the account of this subject in Bayliss' Principles of General Physiol., 1915, 584.

TABLE 1

CONCENTRATION OF PYROGALLOL IN A MIXTURE CONTAINING (AFTER MIXING) 50 PER CENT POTATO JUICE AND 0.93 PER CENT H_2O_2	CHARACTER OF LIGHT FROM THE MIXTURE	CONCENTRATION OF PYROGALLOL IN A MIXTURE CONTAINING (AFTER MIXING) 0.93 PER CENT H_2O_2 AND 0.5 PER CENT BLOOD EXTRACT	CHARACTER OF LIGHT FROM THE MIXTURE
m/4	Faint	m/40	Faint
m/8	Brighter	m/80 to m/8000	Bright
m/16 to m/8192	Bright	m/16000	Fair
m/16384	Fair-faint	m/32000	Faint
m/32768	Very faint	m/64000	Very faint—none
m/65536	None	m/128000	None

TABLE 2

CONCENTRATION OF POTATO JUICE IN A MIXTURE CONTAINING (AFTER MIXING) M/400 PYROGALLOL AND 0.75 PER CENT H_2O_2	CHARACTER OF LIGHT FROM THE MIXTURE	BLUE COLOR WITH TINCTURE OF GUAIAC	CONCENTRATION OF BLOOD EXTRACT IN A MIXTURE CONTAINING (AFTER MIXING) M/400 PYROGALLOL AND 0.75 PER CENT H_2O_2	CHARACTER OF LIGHT FROM THE MIXTURE	BLUE COLOR WITH TINCTURE OF GUAIAC
per cent			*per cent*		
25	Bright	Blue	0.5	Bright	Blue
10	Faint	Blue	0.25	Faint	Blue
5	Very faint	Faint blue	0.05	Very faint	Blue
2.5	None	Faint blue	0.025	None	Blue
0.5	None	Very faint blue	0.01	None	Blue

TABLE 3

CONCENTRATION OF MERCK'S SPECIAL H_2O_2 IN A MIXTURE CONTAINING (AFTER MIXING) M/400 PYROGALLOL AND 50 PER CENT POTATO JUICE	CHARACTER OF LIGHT FROM THE MIXTURE	BLUE COLOR WITH TINCTURE OF GUAIAC
per cent		
0.47	Bright	Blue
0.23	Fair	Blue
0.11	Weak	Blue
0.05	Faint	Blue
0.025	Very faint	Blue
0.012	None	Blue

Each substance must be present in a definite small concentration to produce light. This is m/32000 for pyrogallol, 0.025 per cent for H_2O_2, 0.1 per cent for *dried* blood and 5 per cent for potato juice (i.e., 5 parts pure potato juice to 95 parts water).

Note also from tables 2 and 3 that very often the guaiac reaction can be obtained when, under similar conditions, no light production by pyrogallol takes place.

2. Substances taking the place of oxidase, pyrogallol and H_2O_2.

OXIDASE

Any plant extract containing strong oxidases such as occur in turnips, sweet potatoes, horse radish, etc., or any blood containing haemoglobin will give light with pyrogallol and H_2O_2. Apple juice, which oxidizes tannin, gives no light. Of many invertebrates, including insects, molluscs, annelids, crustaceans and echinoderms, only extracts of Chiton, a tubicolous annelid (*Laonome japonica*), and a polychaete (Nereis sp? —) have thus far been found to give light. The haemocyanin of crab or squid blood does not, nor do extracts of the luminous parts of luminous animals. Among these, bacteria, an annelid, (*Chaetopterus*), a squid (*Watasenia scintillans*), a crustacean (*Cypridina*), a protozoan (*Noctiluca*), a pennatulid (*Cavernularia*) and the fire-fly have been tried.

Among inorganic substances, m/20 $K_4Fe(CN)_6$ gives a bright light, m/10 $FeCl_3$ a fair light and m/40 $KMnO_4$ a faint light. Platinum black, bromine water, m/10 $MnCl_2$, m/10 $FeSO_4$, m/10 $K_2Cr_2O_7$, m/10 $K_3Fe(CN)_6$, m/10 $CuSO_4$, or Fehling's solution at 100° give no light. In greater concentration these substances may oxidize sufficiently to give light as does the mixture of pyrogallol, Na_2CO_3, H_2O_2 and formaldehyde described by M. Trautz (8).

PEROXIDE

Only H_2O_2 will give light with pyrogallol and oxidase. Na_2O_2, BaO_2 or ozonized turpentine do not, although they give a strong guaiac reaction, with the peroxidase of turnip juice.

We must add H_2O_2 to potato juice, which already contains a peroxide whose oxygen may be transferred to guaiac. It is no doubt in too small a concentration to give light with pyrogallol.

OXIDIZABLE SUBSTANCE

Many of the amino- and hydroxy-phenols are rapidly oxidized by potato juice + H_2O_2 but pyrogallol was the only one which gave light. The following were tried and all gave colored oxidation products: phenol, tricresol, pyrocatechin, resorcin, hydrochinon, orcinol, *a*-napthol, ortol, anilin, *o* and *p* toluidin, *a*-napthylamin, guaiacol, paraphenylen-diamine, amidol and pyramidon. Phloroglucin, *β*-napthol and *β*-naphtylamin were not oxidized.

Among the glucosides, vanillin, amygdalin, phloridzin, salicin, saponin and aesculin gave no light with potato juice either at 20° or 60°C.

3. Effect of temperature on the reaction.

A very faint light is produced when pyrogallol + H_2O_2 at 0° is mixed with potato juice at 0°C. The light is much brighter at 5° and quite bright at 10°C. Pyrogallol + H_2O_2 mixed with blood (1 per cent) gives no light at 0°, but a very faint light at 2°C.

High temperatures destroy the pyrogallol oxidase of potato juice. This occurs between 80° and 85° as indicated in table 4. The potato juice was rapidly heated to the temperatures 50°, 55°, 60°, etc., then cooled rapidly in running water. The destruction of catalase, the formation of a precipitate in the potato juice, and the effect on its power to blue guaiac alone, guaiac + H_2O_2 and guaiac + ozonized turpentine are also recorded. Similar results were obtained with tur-

TABLE 4

POTATO JUICE HEATED TO	LIGHT PRODUCTION WITH PYROGALLOL + H_2O_2	BLUEING OF GUAIAC	BLUEING OF GUAIAC + H_2O_2	BLUEING OF GUAIAC + OZONIZED TURPENTINE	EFFECT ON CATALASE. LIBERATION OF O FROM H_2O_2	FORMATION OF PRECIPITATE IN POTATO JUICE
deg.						
50	Bright	Blue	Blue	Blue	+++	None
55	Bright	Blue	Blue	Blue	+	None
60	Bright	Blue	Blue	Blue	None	None
70	Faint	Slight blueing	Fair blueing	Blue	None	Precipitate
80	Very, very faint	Very slight blueing	Slight blueing	Blue	None	Precipitate
85	None	None	None	Blue	None	Precipitate
90	None	None	None	Blue	None	Precipitate
100	None	None	None	Slight	None	Precipitate
100(10 min.)	None	None	None	None	None	Precipitate

nip juice. Blood does not lose its power to oxidize pyrogallol + H_2O_2 with light production or guaiac + H_2O_2 even after ten minutes boiling.

Note from table 4 that the pyrogallol and guaiac oxidases in presence of H_2O_2 are both destroyed between 80° and 85° but that with ozonized turpentine the juice heated to 100° still blues guaiac faintly. The natural peroxide (oxygenase) of potato juice which disappears on standing is destroyed more readily than the peroxidase. Catalase is destroyed between 55° and 60° before a precipitate has appeared, whereas peroxidase is still active after a precipitate has appeared.

4. Effect of KCN, NaOH, and HCl.

In testing the effect of these three substances equal parts of (1 cc. m/160 pyrogallol + 1 cc. 3 per cent H_2O_2) and (potato juice + KCN or NaOH or HCl) were mixed. The table (5) gives also the effect of

TABLE 5

SUBSTANCE	CONCENTRATION IN POTATO JUICE	LIGHT PRODUCTION WITH PYROGALLOL + H_2O_2	LIBERATION OF O FROM H_2O_2	BLUEING OF GUAIAC + H_2O_2	COLOR OF INDICATOR	INDICATOR
KCN	m/20	None	+	None	Yellow	Neutral Red
	m/40	None	+	Very faint blue	Orange	
	m/80	None	++	Fair blue	Orange	
	m/160	None	++	Fair blue	Red	
	m/320	None	++	Fair blue	Red	
	m/640	None	++	Fair blue	Red	
	m/1280	None	+++	Blue	Red	
	m/2560	Very faint	+++	Blue	Red	
	m/5120	Faint	+++	Blue	Red	
	m/10240	Fair	+++	Blue	Red	
	m/20480	Bright	+++	Blue	Red	
NaOH	m/20	None	+	None	Yellow	
	m/40	None	+++	Faint blue	Yellow	
	m/80	Faint	+++	Blue	Yellow orange	
	m/160	Bright	+++	Blue	Red orange	
	m/320	Bright	+++	Blue	Red	Dimethylaminoazobenzol
HCl	m/20	None	None	None	Red orange	
	m/40	None	None	Faint blue	Yellow	
	m/80	None	None	Faint blue	Yellow	
	m/160	Fair	+	Blue	Yellow	
	m/320	Bright	+++	Blue	Yellow	
	None	Bright	+++	Blue	Yellow	

catalase on the blueing of guaiac + H_2O_2, and the color of indicators added to determine the amount of acid or alkali uncombined with protein. Neutral red is red in neutral or acid solution, yellow in alkalies. "Di-methyl" is yellow in neutral or alkaline solution, red in free acid solution.

Note from table 5 that KCN affects the pyrogallol peroxidase in very weak concentration (m/2000) but is not so effective in destroying catalase or preventing the blueing of guaiac.

NaOH and HCl are not so toxic. As we might expect the HCl is the more toxic of the two.

5. Effect of fat solvent anaesthetics.

Saturation of the potato juice or blood with ether or chloroform does not affect its power to produce light with pyrogallol + H_2O_2. A sample of horse radish root extract preserved 45 days under ether gave a good light when mixed with pyrogallol + H_2O_2.

6. Is the oxidase a catalyser?

That oxidases in general are not catalysers but are used up in the reaction has been proven by the work of Kastle (9), who found that the amount of phenolphthalein oxidized is proportional to the quantity of blood present, and of Bach and Chodat (10), who found that the amount of pyrogallol oxidized to purpurogallin is proportional to the amount of peroxidase and also to the amount of H_2O_2 present but independent of the quantity of pyrogallol, providing the pyrogallol is present in excess of the quantity capable of being oxidized by peroxidase and H_2O_2. Melanin formation by tyrosinase is also proportional to the amount of tyrosinase present (11).

The peroxidase, therefore, transfers the O of H_2O_2 to the pyrogallol and the H_2O_2 pyrogallol and peroxidase are all changed, i.e., used up in the reaction. If we represent the peroxidase by P and the pyrogallol by B the reaction must take place as follows:

$$\begin{aligned} P + 2H_2O_2 &= PO_2 + 2H_2O \\ PO_2 + B &= PO + BO, \end{aligned} \tag{1}$$

and not as:

$$\begin{aligned} P + 2H_2O &= PO_2 + 2H_2O \\ PO_2 + 2B &= P + 2BO \end{aligned} \tag{2}$$

Note that in the first equations the peroxidase (P) is used up and in the second equations it acts as a true catalyser and is regenerated again.

That the peroxidase of turnip juice is used up so far as its power to cause light production in pyrogallol is concerned is indicated in the following experiment:

If 1 cc. m/10 pyrogallol + 4 cc. H_2O_2 (3 per cent) is mixed with 5 cc. turnip juice heated to 80° (to destroy the catalase and weaken the peroxidase), a very faint light is produced. A small amount of peroxidase is, therefore, present. The mixture is allowed to stand for 24 hours at 22°C. and then fresh turnip juice added. A good light results, showing that the pyrogallol has not been changed by a small amount of peroxidase when a long time has been allowed for the reaction to proceed. The pyrogallol was turned a light brown by the small amount of peroxidase and this color had not deepened in 24 hours, but did deepen immediately when the additional quantity of peroxidase was added. A small quantity of "enzyme" cannot, therefore, transform an indefinite amount of pyrogallol. A similar result was obtained with a weak haemoglobin solution, 0.1 per cent dried blood extract.

That a large quantity of peroxidase can be used up by addition of successive amounts of pyrogallol (i.e., by "titrating" the peroxidase) can be shown as follows:

To 10 cc. potato juice heated to 60° (to destroy catalase and consequent foaming due to liberation of O from H_2O_2) is added 2 cc. of a mixture of equal parts m/10 pyrogallol and 3 per cent H_2O_2. Light results. When this has disappeared (about 2–3 minutes) 2 more cubic centimeters of the pyrogallol-H_2O_2 mixture is added and a very faint light results. On adding a further 2 cc. no light appears. There is, however, in the final mixture plenty of pyrogallol + H_2O_2 to produce light as may be shown by adding fresh potato juice when abundant light appears. The peroxidase has been completely used up. A similar experiment with blood extract gave a similar result.

SUMMARY

1. Pyrogallol gives off a yellowish white light about equal in intensity to a suspension of luminous bacteria when oxidized by blood or plant juices (oxidases) in presence of H_2O_2.

2. The light is visible in very weak concentrations of pyrogallol, viz., m 32000 or 1 part to 254,000 parts solution. H_2O_2 must be present in at least 0.025 per cent, blood in 0.1 per cent (dried defibrinated blood) and potato juice in 5 per cent (fresh pure juice) to give a just visible light.

3. Of many easily oxidizable hydroxy- and amino-phenols only pyrogallol gives light. $K_4Fe(CN)_6$, $KMnO_4$ or $FeCl_3$ may take the place of the oxidase. Na_2O_2, BaO_2 or ozonized turpentine will not take the place of the H_2O_2. Animal extracts except those of some marine annelids and Chiton do not give light.

4. Light is produced at 0° and a bright light at 10°C. The oxidase of plant juices is destroyed between 80° and 85°.

5. KCN inhibits the light production in very weak concentration, m/1280 to m/2560. NaOH inhibits in n/40 and HCl in n/80 concentration.

6. Ether and chloroform have no effect on the light production.

7. The oxidase is not a true catalyser but is used up in transferring oxygen from H_2O_2 to the pyrogallol.

8. The reaction is compared with the production of light by luminous animals which it mimics very closely.

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THE CORPUSCLES, HEMOGLOBIN CONTENT AND SPECIFIC GRAVITY OF THE BLOOD DURING HIBERNATION IN THE WOODCHUCK (MARMOTA MONAX)

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INTRODUCTION

The fact that a great many physiological and pathological conditions are attended by a variation in the number of corpuscles and in other constituents of the blood, has naturally led to an investigation of the effect of hibernation on the composition of the blood. It is well known that during this period of lethargy, lasting three or four months and even longer, there may be almost continuous sleep and there may have been no ingestion of food or water whatsoever. In consequence there is a loss in the body weight amounting to as much as one fourth. The body temperature may fall from 38°C. to only a few degrees above 0°C. and practically all the vital processes are reduced far beyond that met with in many other conditions that are known to have a marked effect on the blood.

Vierordt (1) was not only a pioneer in the accurate counting of blood corpuscles, but also the first to describe a change in their number during hibernation. In the marmot he found 5,800,000 red corpuscles per c.mm. at the beginning of winter-sleep, while at the end of this period he found only 2,300,000. According to Valentin, (2) he also found that the corpuscles diminished in size as well as in number. Quincke (3) similarly found at the end of hibernation (May 17) in the marmot only 3,500,000 red cells, or 70% of the number found during the summer. Hemoglobin determinations showed only 8.8 per cent or 64 per cent of the normal amount of this pigment. Quincke took the blood from the pulsating heart. He found the erythrocytes to be of the usual form but of various sizes, and some of them contained yellow droplets.

Quincke thought that he saw in the spleen, red bone marrow and liver, during hibernation, abundant evidence of the disintegration of red corpuscles. In the capillaries of the liver, for example he found leucocytes containing iron-holding granules, especially in old marmots. These granules were blackened with sulphide of ammonium. He concluded that the iron from the broken down red cells must accumulate in these organs and later serve as a source of iron in the regeneration of new corpuscles. These pigment granules containing iron were later observed by Carlier (4) to be present in the bile capillaries in the hibernating hedgehog. He also found in the liver and in the large veins coming to the liver, large rounded or oval mononucleated cells, the cytoplasm of which often contained iron-holding pigment. He believed that these large cells were macrophages which had wandered into the liver after having absorbed pigment resulting from the destruction of erythrocytes. Unlike Quincke, however, he does not think that this pigment is stored in the liver for future use, but that it simply accumulates there owing to the sluggish state of the excretory function of that organ during hibernation. Later the pigment is most likely excreted as usual, since he found that the liver did not appear to be a blood-producing organ after the animal wakes up from winter-sleep.

The red corpuscles were also counted by Dubois (5) in a number of marmots at various times from November to May. His table of results shows that with the progress of hibernation there is a slight increase in the number of erythrocytes, both in arterial and in venous blood (the blood being from the larger vessels), until towards the end of this period, at which time there is a decrease from over 5,000,000 to less than 3,000,000 per c.mm. while the animal is still torpid, and to about 2,000,000 when the animal wakes up. In two cases the exact figures may be given with profit.

ANIMAL	DATE OF ENUMERATION	CONDITION OF ANIMAL	RECTAL TEMPERATURE—C	ARTERIAL BLOOD	VENOUS BLOOD	PORTAL BLOOD
I	February 12...	Torpid	5.6	5,320,000	5,765,000	5,675,000
	February 15...	Awake	34.2	4,730,000	4,905,000	5,885,000
II	May 11.......	Torpid	15.0	2,930,000	2,540,000	
	May 11.......	Awake	35.0	2,070,000	2,530,000	

The extremely low figures for the animal examined on May 11 were checked up by hemoglobin determinations, which showed only 8.5

per cent hemoglobin in the arterial blood and 7.50 per cent to 7.75 per cent in the venous blood while the animal was asleep with a rectal temperature of 15°C. When this same animal woke up on the same day and the body temperature had increased to 35°C. there was still less hemoglobin, especially in the arterial blood. The figures given are 7.25 per cent to 7.50 per cent for both the venous and the arterial blood. The hemoglobin determination thus checks well with the "blood count," since at the beginning of hibernation there was about double this amount of hemoglobin.

It appears from these figures, therefore, that there is a distinct decrease in the number of red corpuscles when the animal wakes up both during mid-hibernation and at the end of this period.

The gradual increase in the number of erythrocytes at the first progress of hibernation which Dubois noted, he attributes to an increase in the specific gravity of the blood due to loss of water, for he found the density of whole arterial blood of the hibernating marmot to be 1.071 and defibrinated arterial blood, 1.0654. The corresponding figures for the awake marmot are 1.046 and 1.0525. The great decrease in the number of red corpuscles at the end of winter-sleep he takes to indicate that there is an actual destruction of these cells.

Polimanti (6) gives the following table as the results of the enumeration of the red corpuscles of four marmots by means of the Thoma-Zeiss apparatus:

ANIMAL	RECTAL TEMPERATURE—C	CONDITION OF ANIMAL AT THE TIME OF ENUMERATION	BLOOD FROM CAROTID ARTERY	BLOOD FROM JUGULAR VEIN	BLOOD FROM PORTAL VEIN
I	10.5	Dormant 15 days	5 250,000	5,450,000	5,620,000
II	7.5	Dormant 8 days	5,350,000	5,565,000	5,700,000
III	9.0	Dormant 20 days	5,400,000	5,610,000	5,780,000
IV	10.8	Dormant 30 days	5,500,000	5,760,000	5,850,000
I	36.0	Awake, July	4,540,000	4,900,000	5,150,000
II	36.5	Awake, at the end of hibernation	4,500,000	4,950,000	5,200,000
III	36.4	Awake, October	4,650,000	5,000,000	5,150,000
IV	36.7	Awake, September	4,700,000	5,100,000	5,200,000

His figures on the hemoglobin content of the blood of four marmots during winter-sleep and again when awake show an average per cent of 18.62 in carotid blood, 11.06 in jugular blood and 25.82 in portal blood during hibernation, and 15.905, 15.46 and 21.22 respectively when awake and active. The blood from the jugular vein is the only case where during hibernation there is less hemoglobin than during the waking state. Besides indicating a greater percentage of hemoglobin in the arterial and the portal blood during hibernation, and thus in general verifying the corpuscle count, these figures show an increasing quantity of hemoglobin as you go from the jugular vein to the carotid artery and finally to the portal vein. This, he thinks, indicates that there is an unequal distribution of the red corpuscles in the various vascular zones. His table given above shows that in this particular the carotid and jugular blood ought to be interchanged in order to agree with the hemoglobin determinations. Incidentally it might be noted that the erythrocytes are most numerous in the portal blood. Upon the theory that the increase in the number of red cells following exercise and other factors that increase blood pressure is due to bringing into circulation more of the corpuscles that tend to settle down in the splanchnic area during a slower circulation (7), one might expect even a comparatively greater number of erythrocytes in the portal blood during such a sluggish circulation as is seen in hibernation.

Polimanti's figures are in general agreement with those of the previous authors, except that the decrease at the end of winter-sleep is not nearly so great as reported by them. He, like Dubois, considers the slight increase during the progress of hibernation as being only apparent and as explainable by the increase in the specific gravity of the blood. He found in the case of three marmots that the density of the blood on November 15, 1895, during the normal waking state was 1.045, 1.050 and 1.037. On March 10, 1896, the corresponding figures were: 1.080, 1.075 and 1.065. At this latter date the animals were hibernating. That the blood loses water during hibernation is indicated by the work of Aeby (8) who analysed the various tissues and organs of the marmot for the water and the ash contents during the early part of hibernation and again during the latter part of this period. He found the blood in the early part of winter-sleep to contain 83 per cent water, 17 per cent dried solids, 0.87 per cent ash in the fresh blood and 5.11 per cent ash in the dried solids. In the latter part of the dormant period the corresponding figures are 78.63 per cent water, 21.37 per cent dried solids, 0.91 per cent ash in the fresh blood and 4.26 per cent ash in the dried solids. Dubois obtained venous and arterial blood from the femoral vessels and dried in at a temperature of 100°C. over sulphuric acid and found 84.51 per cent water in the arterial blood and 83.70 per cent water in the venous blood of an active marmot with a rectal temperature of 35°C. In a dormant marmot with a rectal temperature of 11°C., the corresponding figures obtained were 79.76 per cent water in the arterial and 80.23 per cent in the venous blood. This is an average loss of about 4.11 per cent. Polimanti similarly observed a decrease in the water content of the blood of the hibernating marmot amounting to about 4.6 per cent.

In regard to the white corpuscles, Quincke found that they were present in fair quantities during hibernation and were not practically absent as was stated by Valentin. According to Quincke, Valentin explained this reduction in the white cells as being due to the absence of lymph, which he thought conveyed the leucocytes into the blood stream. A table of the actual number of white corpuscles found by Dubois (wrongly attributed to Quincke by Polimanti, p. 186) in the marmot shows that in the carotid, jugular and portal blood of a hibernating animal with a rectal temperature of 8.6°C. there were respectively 4,000, 7000 and 9000 leucocytes; while in an active animal with a rectal temperature of 34.2°C. the corresponding numbers were 7000, 12,000 and 9000. It appears from this that the arterial blood is especially poor in white corpuscles during winter-sleep. The increase in the number of white cells in the blood vascular system generally and especially in the veins in the active state is explained by Dubois as being due to the greatly increased circulation of peritoneal lymph at the time of waking up. The fact that there is no such increase in the portal blood at this time he thinks is probably due to the fact that the large lymphatic trunks about the portal vein do not empty into the portal vein, but course directly into the liver.

Carlier (p. 97) found that in the hedgehog during hibernation there is a great increase in the number of wandering cells in the connective tissue of the mucous and submucous coats of the stomach and in some other places. He thinks that these cells are white corpuscles that have migrated from the circulating blood. He cites in support of this idea the fact that normally during the active state the blood of this animal contains from 18,000 to 20,000 leucocytes per c.mm., but the number falls suddenly at the very commencement of winter-sleep to from 1000 to 8000. At the same time he noted that the red corpuscles show, if anything, rather an increase in number. Polimanti similarly showed that in the marmot there is a marked decrease in the number of circulating white corpuscles, which he also explains as being due to diapedesis which always occurs during hibernation and which is also responsible for the large amount of lymph found in the peritoneal cavity in winter-sleep. His figures for the same animals and under the same conditions as those mentioned in connection with his red count, are strictly in agreement with those given by Dubois and show an average of 3850, 6898 and 8663 per c.mm. of blood from the carotid artery, jugular vein and portal vein respectively, during hibernation; while the corresponding figures for various periods of the active stage are: 6535, 11,148 and 9020.

All the data that have been found so far on this subject seem to warrant the conclusion that during hibernation in warm blooded animals there is a great reduction in the number of circulating leucocytes. In the European marmot, which has been investigated most thoroughly, this reduction amounts to about one half.

Very little work has been done on differential counting of the white corpuscles during hibernation. Argaud and Billard (9) induced dormice to hibernate in the summer by depriving them of food. After ten days of such sleep, they found only a few white cells in the blood and these were of the mononuclear type.

It is interesting in this connection to note that, while there is a great deal of disagreement in the results of blood examination during long inanition in man and other animals, many authors report striking alterations in the number and formula of the leucocytes, indicating in general that there is a decrease; a slight absolute loss of both red corpuscles and hemoglobin, although these elements seem to be very resistant; a tendency towards a concentration of the blood and hence an increase in the specific gravity due to the loss of water, with a slight relative increase in hemoglobin and erythrocytes. Upon refeeding there is a relative decrease in the hemoglobin, red corpuscles and specific gravity due to the acquisition by the blood of water faster than other elements. Yet a month of fasting (water being allowed) by man may show surprisingly little or no change in these respects (10).

PRESENT INVESTIGATION

The work reported here is based upon observations carried out during the last three years on the woodchuck (Marmota monax), a rodent which hibernates profoundly three or four months each year without having anything to eat or drink during this time. These animals were captured uninjured in this vicinity (Ithaca, N. Y.), and were kept in the artificial burrows designed by Dr. Simpson of this laboratory and described in detail elsewhere (11). Many of the animals were used a few days after being captured. While in captivity they were supplied with abundant food and water. Those investigated while dormant were obtained usually in September and fed till hibernation set in. The conditions of the artificial burrows are such that there is no reason to believe that the animals kept in them will not be representative of those in the natural burrows. Some of the animals were kept till spring and allowed to wake up. A number of these animals were investigated before they had recommenced to feed and others were fed for some time and then examined. A number of animals were also obtained fresh from the field in the early spring after feeding had commenced. A much larger number of animals would have been desirable especially during some of the stages, but due to the escape of 32 animals of one series through a hole accidentally or maliciously made in the metal wall of the inclosure at a late date in autumn when no more could be obtained, and the death of about 15 of another series during the fall and winter, the number of animals was unintentionally decreased.

METHOD.

The active animal was rapidly anaesthetized with ether by being put into a small box in which ether had been placed some time previously. The onset of anaesthesia was rapid and not attended with marked struggling. Since the duration of the ether anaesthesia before the blood determinations were made was, as a rule, less than twenty minutes, there ought to be but little error in the relative results from these disturbing factors. The dormant animal was either not given any anaesthesia at all, or was given chloretone one hour before the operation and was kept undisturbed in a basket of straw or, if practicable, in its habitual burrow until the operation was commenced. It was found necessary to give, even to the active animal, twice the dose prescribed for the rabbit. In order not to introduce too much liquid into the rectum and thus dilute the blood appreciably, 2 cc. of 50 per cent alcohol containing 0.3 gram of chloretone was given per rectum for each kilogram of body weight. This did not disturb the animal noticeably and the blood determination from such animals compared well with those not given any anaesthetic. Chloretone was given in order not to disturb the animal by the preliminary operation involved in exposing the vessels, since the object was to determine the condition of the blood in the dormant animal, and since after the blood determinations, other experiments were frequently planned which demanded an undisturbed animal. The use of a little cocaine as a local anaesthetic while isolating the vessels was also resorted to in some cases.

Semi-dormant animals were either given chloretone, aided by cocaine as a local anaesthetic, or ether. This point will be discussed in connection with the results.

Blood was obtained from the carotid artery (in a very few cases from the femoral artery) by means of a glass cannula and a short rubber tube. The cannula and rubber tube were washed out by allowing a little blood to escape and then from 1 to 2 cc. of blood was collected in a small paraffined vessel. The pipettes of the Thoma-Zeiss haemocytometer and the Gowers-Haldane haemoglobinometer were then filled in rapid succession and four cover glass smears were made. These smears were later stained with Wright's blood stain. The cannula and rubber tube were again flushed out with blood and a fresh supply amounting to nearly 6 cc. was collected in a small specific gravity bottle. The stopper was carefully inserted so as to inclose no air bubbles and the bottle wiped free from blood on the outside with a moist cloth. The weight of the blood was compared with an equal volume of distilled water at the same temperature. Thus a very accurate determination of the specific gravity was possible.

In case of the erythrocytes at least 400 and in case of the leucocytes at least 800 squares were counted, and at least two samples were taken from the pipette. The same counting chamber, cover glass and pipettes were used through the entire series, and furthermore the cover-glass was placed with the same side down and as nearly in the same position as could be judged by the outline of the square coverglass which had been scratched on the counting chamber. With these and the other usual precautions necessary to the obtaining of reliable results, together with the advantage of having an ample supply of fresh blood direct from a large artery and plenty of time to fill the pipette accurately, the figures obtained are believed to be free from serious error.

The Gowers-Haldane clinical method of hemoglobin determination was considered sufficiently accurate under the conditions, and hence the results are really in percentages of normal oxygen capacity (18.5 volumetric per cent) of man, which usually means about 14 per cent hemoglobin. Since no standard for the woodchuck exists, only the relative amount of hemoglobin is indicated.

RESULTS

The results are placed in the tables below.

Group I. Before hibernation. Animals active

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R. T.C.	R. B.W.	White	Red		
1913								
November 22....	2–SI	♀	37.2	2440	18,440	6,229,600	109	1.0591
December 13....	14–SI	♂	35.8	2650	12,132	5,864,000	100	1.0597
1915								
July 8...........	5–SIII	♀	36.1	2757	14,000	7,748,000	118	1.0639
July 9...........	6–SIII	♀	38.4	2464	12,300	5,542,000	100	1.0569
July 20..........	12–SIII	♂	36.7	2690	10,800	5,268,000	90	1.0555
July 29..........	17–SIII	♀	36.7	2760	3,900	5,464,000	94	1.0540
August 2.......	19–SIII	♀	36.7	2771	10,300	6,106,000	110	1.0627
August 5.......	20–SIII	♂	38.5	3190	11,700	6,263,000	110	1.0625
August 20.......	26–SIII	♀	38.3	3130	13,500	6,580,000	108	1.0641
August 24......	28–SIII	♀	37.2	2284	15,500	6,075,000	100	1.0558
August 26.......	29–SIII	♀	36.9	2230	13,000	7,418,000	114	1.0633
November 6.....	37–SIII	♀	36.5	1725	18,450	7,298,600	114	1.0606
November 20....	39–SIII	♂	36.0	1730	9,950	5,150,000	90	1.0566
November 23....	40–SIII	♀	37.0	1622	6,550	5,942,000	98	1.0577
Average.......	....	....	......	2460	12,180	6,210,586	103.9	1.05946

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group II. During hibernation. Animals dormant.

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R. T.C.	R. B.W.	White	Red		
1914								
February 3......	5–SI	♂	8	2225	6,300	5,357,000	99	1.0587
February 28.....	7–SI	♀	12	2475	7,600	5,641,000	95	1.0599
March 14.......	9–SI	♀	11	1375	5,432	5,976,700	100	1.0594
1915								
December 18....	42–SIII	♀	9	2100	4,360	5,832,000	100	1.0554
December 28....	45–SIII	♀	10	2046	10,050	7,949,000	115	1.0647
1916								
February 7......	48–SIII	♂	6	813	800	6,678,000	100	1.0600
February 26.....	51–SIII	♀	7	1635	7,200	6,928,000	108	1.0581
March 4........	52–SIII	♂	10	1203	5,050	6,768,000	102	1.0565
March 6........	53–SIII	♂	12	1849	6,800	7,796,000	114	1.0628
Average.......	....	....	......	1747	5,955	6,547,300	103.7	1.0595

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group III. During hibernation. Animals sluggish or semi-dormant

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB; (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R.T.C.	R.B.W.	White	Red		
1915								
December 21....	43–SIII	♂	19	2525	34,000	7,606,000	118	1.0654
1916								
February 8......	49–SIII	♂	23	1615	22,250	7,524,000	114	1.0608
April 1..........	55–SIII	♂	24	1355	19,400	7,528,000	110	1.0625
Average.......	....	....	......	1832	25,217	7,552,667	114	1.0629

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group IV. After hibernation. Animals awake and active. Nothing to eat or drink.

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R.T.C.	R.B.W.	White	Red		
1914								
March 18.......	11–SI	♀	36	1325	12,266	6,666,000	114	1.0600
March 25.......	13–SI	♂	34	2175	8,600	6,556,000	116	1.0628
March 28.......	14–SI	♂	35	1725	4,767	6,934,000	115	1.0648
1916								
February 25.....	50–SIII	♀	35	2936	7,750	7,363,000	116	1.0659
April 3..........	56–SIII	♂	34	2210	9,650	7,338,000	109	1.0612
Average.......	....	....	......	2074	8,607	6,971,400	114	1.06294

R. B. W., Reduced body weight. R. T., Rectal temperature.

Group V. After hibernation. Animals awake and active. Fed.

DATE OF OBSERVATION	ANIMAL				CORPUSCLES		HB. (RELATIVE)	SPECIFIC GRAVITY
	Number	Sex	R. T.C.	R. B.W.	White	Red		
1914								
April 5..........	15–SI	♂	36	1803	9,067	5,506,000	92	1.0539
1915								
May 18.........	14–SII	♀	37.7	1550	7,200	4,880,000	82	1.0489
1916								
April 22.........	59–SIII	♂	36	1775	5,400	5,176,000	90	1.0515
April 24........	60–SIII	♀	37.5	1175	13,550	4,936,000	85	1.0493
April 28.........	61–SIII	♀	35	1511	11,700	7,416,000	115	1.0611
May 4..........	64–SIII	♀	37	1695	5,800	4,644,000	75	1.0480
May 10........	66–SIII	♂	37	2420	10,800	5,328,000	100	1.0569
May 12.........	67–SIII	♀	38	1690	17,600	5,094,000	84	1.0491
May 13.........	68–SIII	♀	38.5	1810	11,450	5,188,000	85	1.0491
Average...........................				1714	10,285	5,352,000	89.8	1.0520
Average-Excluding No. 61–SIII............					10,108	5,094,000	86.6	1.0508

R. B. W., Reduced body weight. R. T., Rectal temperature.

An examination of the above results shows that in fourteen woodchucks, in the active state, during the summer and autumn, the number of red corpuscles per c.mm. varies from 5,150,000 to 7,748,000, with an average of 6,210,586. The relative amount of hemoglobin varies from 90 to 118, with an average of 103.9. The specific gravity ranges from 1.0540 to 1.0641, with an average of 1.0595. If the specific gravity of the blood of seven other animals, used for other experiments, be considered, the limits in a total of twenty-one animals are 1.0540 and 1.0658 with practically the same average, or 1.0596. The individual variations in the erythrocytes, hemoglobin and specific gravity usually run more or less parallel in this as well as in the other groups. The white corpuscles range from 3900 to 18,450, with an average of 12,180. Only in one case is there any exceptional departure from the usual variations characteristic of a leucocyte count.

From the figures in the second group of nine dormant animals which were examined at various intervals during hibernation (December to March) it is evident that in the case of the red cells there are about the same limits to the variations and an average of 6,547,300, or about 5 per cent more than during the active state before hibernation; while the average specific gravity and the average amount of hemoglobin remain practically the same as in the first group. By including three more animals, the specific gravity of whose blood was examined while dormant, the average is raised slightly or to 1.0599. The fact that the average body weight in this group is only 1747 grams as compared with 2460 grams in the first or active group does not mean that the dormant animals were much smaller and hence much younger. There is in the dormant group only one especially young animal (as judged from its weight of only 813 grams) and in this case the specific gravity and number of red corpuscles are above the average for the group, while the hemoglobin is less than the average by only slightly more than the probable error. It must be remembered that during hibernation the animal loses at least one fourth of its weight. In short, the great increase in the specific gravity and in the amount of hemoglobin of the blood during winter-sleep as stated by Dubois and Polimanti in regard to the European marmot, does not apparently take place in the woodchuck. Nor is there a very marked increase in the red corpuscles.

An entirely different picture is, however, presented by the disposition of the white corpuscles. The table shows that there is a variation from 800 to 10,050, with an average of 5955. With the exceptions of the two cases which mark the extreme variations, the individual counts are surprisingly close to the average for the group. Thus during hibernation there is in the woodchuck, as in other animals investigated, an enormous decrease in the number of circulating leucocytes. In this case, as in the European marmot, the number falls to about one half the number found during the summer. This has been explained by Dubois, Carlier and Polimanti as being due to diapedesis. In this connection might be mentioned a theory advanced by Hansemann (12) who, upon examination of the various organs of animals killed while hibernating, found no evidence of cell division. He concluded that cell division normally occurs as a result of the wearing out of the tissues. When activity is greatly reduced, as in winter-sleep, the stimulus is gone and there is no cell division. In accordance with this theory, there might be a decrease in the production of leucocytes, which would also contribute to the reduced number of circulating white corpuscles.

A few semi-dormant animals were examined during the hibernating period and found to present such a marked contrast to the more torpid ones that they have been placed in a separate group. Since there are only three individuals not much can be based upon them, but it did not seem justifiable to include them with the more dormant ones. These animals had a rectal temperature from 19° C. to 24°C. No. 43 was beginning to hibernate. No. 49 was either going back to sleep or coming out of it. No. 55 was waking up from winter-sleep. The red corpuscles are increased 1,000,000, or more, and the hemoglobin and specific gravity likewise show the maximum average figures of 114 and 1.06294 respectively. Still more marked is the large number of white corpuscles which average 25,217, or more than double that of any corresponding figure. Since these animals were moving about, it was necessary to employ some anaesthetic. Ether was used on No. 43. While anaesthesia set in rapidly and had only been continued ten minutes before the blood sample was taken, it was considered probable that the high figures were in some way, at least partly, due to the ether. The next animal, No. 49, was given chloretone with as little disturbance as possible; but all the figures were still high. On the third animal, No. 55, ether was tried again, with somewhat similar results. It appears that in this transitional stage, there is an enormous number of leucocytes circulating in the blood. It might be that there is some irritation or struggle within the organism, such as resistance against the cold, during the transition which causes leucocytosis and a dislodging of corpuscles, both red and white, which had settled out of circulation. The high specific gravity would suggest that enough activity is going on to cause a loss of water by the blood, somewhat the same as apparently occurs after the animal becomes fully awake and active and has no access to food or water.

In the fourth group there are included those animals which have become fully awake after hibernation and have not had anything to eat or drink. They had been awake from two days to two weeks. Here again the group is rather limited, involving only five animals, but the results are strikingly uniform and opposed to what one might expect from the literature. The red corpuscles average nearly 7,000,000, which is fully 500,000 more than was found during torpor, and a little more than 500,000, or 8 per cent, less than was found in the semi-dormant animals. The specific gravity and the amount of hemoglobin show the same average as was found in the third group, and suggest a concentration of the blood by the loss of water due to increased activity and a much greater difference between the body temperature and the temperature of the surroundings. Thus it has been fully demonstrated by the extensive observations of Jones (13) that in the human subject exercise causes a high specific gravity of the blood. The woodchuck shows no evidence of the great decrease to one-half the normal number of erythrocytes when the animal wakes up from lethargy, such as is described by Vierordt, Quincke and Dubois in connection with the European marmot. This decrease, then, is not always characteristic of hibernating animals when they first wake up, for exactly the opposite condition prevails in the woodchuck.

The number of white corpuscles is very uniform and they are somewhat more numerous than during dormancy, but not as numerous as during the active period in summer and autumn, while they are only one third as numerous as in the third or sluggish group.

Finally, there is the series of nine animals which have had access to food and water for one to at least six weeks either in confinement or in their natural habitat. The red corpuscles show a sudden drop of over one and a half million, or over 20 per cent, as compared with the animals that have not been fed. The hemoglobin similarly shows a decrease of over 20 per cent. The specific gravity likewise decreases to 1.[illegible] indicating that the blood has been diluted from having taken up water faster than the other constituents. It is possible that the sudden decrease in the red corpuscles noted by several authors in the

European marmot may be due partly, at least, to the same cause. The results obtained from the woodchuck are very uniform with a single exception, No. 61. This animal was caught by some boys one night in a trap. It was taken to the public school the next day and played with till evening when it was brought to the laboratory. It was placed in a box containing food and water. A dark secluded box communicates with the feed box and into this the animal soon found its way. It was operated upon the next day and from the appearance of the intestinal contents it was evident that it had not eaten any of the food in the box. It was impossible to judge as to the drinking of water, since there were other woodchucks in the same box. There are only 64 grams of feces in the alimentary tract and this was unusually solid. As a rule, there is about 200 grams in such animals. It is, therefore, very probable that the struggling and excitement of the previous day had concentrated the blood by causing an excessive loss of water. However, it is included in the figures already mentioned. The average for Group V, excluding this questionable one, is also given. Excluding animal No. 61, the red corpuscles vary within the comparatively narrow limits of 4,644,000 and 5,506,000, with an average of 5,094,000, or about 18 per cent lower than is found later in the summer. Undoubtedly with the onset of warm weather these low figures will approach those found in Group I. The hemoglobin ranges from 75 to 100, with an average of 86.6, while the specific gravity has fallen to an average of only 1.0508, and within the limits of 1.0480 and 1.0569.

The white corpuscles in this group have increased from 8607 (in Group IV) to 10,285 or until they are nearly as numerous as later in the year.

Undoubtedly most of the variations (except those in the leucocytes) are dependent to a large extent upon the concentration of the blood and redistribution of the corpuscles. Sufficient data are not yet available on the changes in blood volume to determine what absolute changes are incident to hibernation.

The size of the red corpuscles does not vary appreciably as will be seen from the following tabulated averages of the measurement of the diameter of 100 corpuscles from each of 30 animals. Each group of 10 animals consists of 5 males and 5 females.

BEFORE HIBERNATION August-December	DURING HIBERNATION December-March	AFTER HIBERNATION March-May
Microns	*Microns*	*Microns*
7.36	7.37	7.39
7.34	7.33	7.37
7.28	7.27	7.36
7.27	7.24	7.29
7.25	7.23	7.27
7.24	7.22	7.23
7.23	7.22	7.19
7.18	7.18	7.10
7.14	7.15	7.06
7.01	7.03	7.01
Average....7.230	7.224	7.227

The above measurements were made on the stained film preparations by means of a filar micrometer ocular. Only perfectly shaped corpuscles encountered in the thinnest parts of the films were utilized. Care was taken to select portions of the film in which the corpuscles were well spread and which had dried quickly as judged by the preservation of the blood plates. Since four films from each animal were available, the perfect erythrocytes found within certain limits in the field were taken, commencing at one side of the area and going towards the other, in a regular order until twenty-five corpuscles had been measured. This procedure was repeated on the other three duplicate preparations. The average of all the one hundred measurements was taken as the size of the corpuscles in that particular animal.

In each animal the dried red corpuscles usually varied from 5.8μ to 9.2μ in diameter. Only a very few cells, however, were at or near these extremes.

The results of a differential count of the white corpuscles, utilizing again the stained films, are given in the following table. At least 500 corpuscles were examined in each case.

The percentage of lymphocytes in the group of eight dormant animals varied greatly (from 7.8 per cent to 42 per cent), as did also that of the neutrophiles (from 54.7 per cent to 90.3 per cent). The other groups showed less individual variations in these particulars. In the group of five active animals examined after hibernation but before they had commenced to feed there is an unusually large individual variation in the relative number of eosinophiles (from 0.2 per cent to 18.4 per cent) and a rather high average (11.4 per cent).

ANIMAL	LYMPHOCYTES (LARGE AND SMALL)	MONONUCLEAR AND TRANSITIONAL LEUCOCYTES	POLYMORPHO-NUCLEAR LEUCOCYTES		
			Neutrophilic	Eosinophilic	Basophilic
Before hibernation (August–November), animals active					
19–SIII	34.2	6.7	57.6	1.2	0.3
20–SIII	33.4	6.6	56.6	3.4	0.0
26–SIII	24.8	5.8	64.7	4.3	0.4
28–SIII	21.6	3.6	71.4	3.2	0.2
29–SIII	39.7	9.0	42.2	8.7	0.4
37–SIII	25.9	5.2	62.4	6.5	0.0
39–SIII	33.1	7.9	56.4	2.6	0.0
40–SIII	17.9	3.8	71.0	7.1	0.2
Average......	28.8	6.1	60.3	4.6	0.2
During hibernation (December–March), animals dormant					
5–SI	25.6	4.4	66.1	3.5	0.4
7–SI	34.1	4.1	54.1	7.0	0.7
9–SI	42.0	8.9	36.3	12.3	0.5
45–SIII	11.5	6.0	76.7	5.6	0.2
48–SIII	13.4	8.1	76.6	1.9	0.0
51–SIII	7.8	3.5	84.7	4.0	0.0
52–SIII	10.0	5.8	79.4	4.8	0.0
53–SIII	19.7	6.2	67.5	6.6	0.0
Average......	20.5	5.9	67.7	5.7	0.2
During hibernation, animals sluggish or semi-dormant					
43–SIII	17.2	8.5	68.9	5.4	0.0
49–SIII	26.8	9.0	58.1	6.1	0.0
55–SIII	28.3	4.7	56.5	9.0	1.5
Average......	24.1	7.4	61.2	6.8	0.5
After hibernation (February–April) animals active; nothing to eat or drink					
11–SI	37.7	13.9	28.5	19.4	0.5
13–SI	21.9	7.4	54.1	16.4	0.2
14–SI	30.3	5.9	45.8	17.3	0.7
50–SIII	42.2	4.4	52.8	0.2	0.4
56–SIII	43.6	8.8	43.3	3.5	0.8
Average......	35.1	8.1	44.9	11.4	0.5

ANIMAL	LYMPHOCYTES (LARGE AND SMALL)	MONONUCLEAR AND TRANSITIONAL LEUCOCYTES	POLYMORPHO-NUCLEAR LEUCOCYTES		
			Neutrophilic	Eosinophilic	Basophilic
After hibernation (April–May), animals active, fed					
15–SI	39.3	6.3	43.9	10.2	0.3
59–SIII	27.1	5.4	63.3	3.6	0.6
60–SIII	27.6	5.8	50.2	15.9	0.5
61–SIII	19.4	4.0	71.5	5.1	0.0
64–SIII	46.6	6.3	40.8	6.3	0.0
66–SIII	29.8	9.4	51.3	7.8	1.7
67–SIII	21.9	10.3	57.3	7.6	2.9
68–SIII	42.7	6.3	39.2	10.9	0.9
Average......	31.8	6.7	52.2	8.4	0.9

It is evident that in hibernation in the woodchuck there is no such inversion of the leucocyte formula as was reported by Argaud and Billard to occur in the dormouse when induced to hibernate. On the contrary, there appear to be relatively fewer lymphocytes circulating during dormancy and semi-dormancy than during the active state, either before or after hibernation. This decrease is undoubtedly due to a much slower circulation of lymph in this torpid state.

A comparison of the group of animals that had been fed with those that had not been fed but were awake after hibernation, bears out the general statement that in herbivorous animals there is no digestion lymphocytosis.

Blood plates (thrombocytes) are very abundant at all seasons of the year.

SUMMARY

1. In the summer and autumn there are in the arterial blood of the woodchuck on an average, in round numbers, about 6,200,000 red corpuscles, 12,000 white corpuscles, 104 per cent hemoglobin (Gowers-Haldane standard), and a specific gravity of 1.0595.
2. There is very little change in the number of red corpuscles, amount of hemoglobin and specific gravity of the arterial blood in the dormant woodchuck during hibernation. The figures for the erythrocytes do show an increase of about 5 per cent.
3. The number of circulating white corpuscles decrease to about one half in the torpid animal during hibernation.
4. The cellular elements (erythrocytes and leucocytes), the amount

of hemoglobin and the specific gravity increase greatly in animals which during the hibernating period are only semi-dormant and have an intermediate body temperature. The white corpuscles are four times as numerous as in the dormant animal and twice as abundant as in the active animal before hibernation.

5. After the animal wakes and becomes active, but before it has access to food, there is the same high specific gravity and large amount of hemoglobin as is found in the semi-dormant animals. The red corpuscles, however, are about 8 per cent lower, but this is still about 6 per cent higher than during the torpid state. The leucocytes are sub-normal, though somewhat more numerous than during profound lethargy.

6. There is a decided decrease, amounting to about 20 per cent, in the number of red corpuscles and the amount of hemoglobin after the awakened animal has commenced to eat and drink. The specific gravity greatly decreases also, suggesting that these variations are largely due to the relative concentration of the blood. The white corpuscles, on the other hand, increase to nearly normal.

7. The red corpuscles do not change in size during hibernation.

8. The leucocyte formula is not inverted with respect to the relative number of mononuclear and polymorphonuclear forms. There is, if anything, a decrease in the mononuclear types during winter-sleep due to a relatively greater decrease in the number of circulating lymphocytes.

It is with great pleasure that I acknowledge the credit due Dr. Sutherland Simpson for the opportunity of carrying on this research and for the suggestions and encouragement received at all times. The valuable help rendered by Gertrude B. Rasmussen is also worthy of mention.

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THE DIASTASE IN THE SALIVA OF THE OX

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INTRODUCTION

The importance of the digestive action of the saliva of man on starch was long underestimated, but it is now known to be of considerable importance. Its importance is due to the rapidity of the action and to its continued activity after food reached the stomach. It was formerly thought that the action was checked at once on entrance of food into the stomach. We now know that this is not the case, but that the food collects in the fundus of the stomach in a large mass, in the interior of which the reaction is very faintly acid. The optimum conditions for ptyalin action prevail and for a half hour or longer, depending on the size of the meal, the ptyalin may continue to digest the starches. Some workers are also of the belief that the presence of saliva, or its digestive products, may also act as a stimulus to the secretion of gastric juice.

The question of the presence of a starch splitting enzyme in the saliva of the ox is a disputed one. Ellenberger (1) states that Speicheldiastase (ptyalin) is found in the saliva of man and animals, except carnivora. Human saliva is the most active, next the pig, solipeds slight, ruminants still less, and carnivora very little or none. Ellenberger (2) also believes that in animals it is a specific product of the salivary glands. He states that it attacks raw starch with difficulty but cooked starch easily. On a diet of raw starch (corn) and after masticating the food for one to three minutes, Ellenberger (3) states, sugar can be demonstrated in the oesophagus. He also thinks the action is continued for some time in the stomach.

Meade Smith (4) believes that in ruminants, starch conversion takes place both in the mouth and rumen. Fred Smith (5) does not accept these views and states that the action is very feeble or entirely absent.

Mills (6) states that the saliva of the ox has a very feeble diastatic power. It was therefore the object of this investigation to demonstrate the presence or absence of a diastatic enzyme in the mixed saliva of this animal.

METHOD OF COLLECTING SALIVA

Various drugs have the property of stimulating the secretion of saliva. Chief among these drugs is pilocarpine which, when given subcutaneously in doses of ½ to 1 grain, produces a marked flow of saliva in this animal. It occurred to the writer that pilocarpine would be a very useful agent to employ, as an aid in collecting the saliva, provided it did not in any way influence the diastatic action of the saliva.

Accordingly, preliminary experiments were carried out to determine the action of pilocarpine upon the diastatic action of human saliva, the normal action of this saliva being well understood. It was found that pilocarpine does not inhibit the diastatic action of human saliva, when added to it. Neither does it interfere when taken (via mouth) in large enough doses to cause a marked flow. However, when pilocarpine in aqueous solution is added to a 1 per cent solution of cooked starch and when no saliva is present, it exerts an action similar to that of saliva. Our experiments show that as small an amount as 0.00081 gram will reduce Fehling's solution and when this amount is incubated with 25 cc. of 1 per cent starch, the blue color with iodine disappears in about 20 minutes, and the starch solution will become clear in fifteen minutes, and will show increased reduction of Fehling's solution. It differs from ptyalin in that its action is much slower when used in this amount, but the rate of action can be increased by increasing the amount of the drug. When ½ grain in 25 cc. of distilled water was added to 25 cc. of 1 per cent starch solution, the blue color with iodine disappeared within ten minutes, and the solution was clear in about five minutes. Pilocarpine because of these properties could not be used in our work for stimulating salivary secretion. Enough of the drug can be excreted in the saliva to produce a marked diastatic action which might lead to wrong conclusions.

When ½ grain of pilocarpine is administered subcutaneously to an ox, a marked secretion of saliva occurs in about ten minutes. This saliva contains a small amount of pilocarpine and will produce the characteristic action of this drug upon starch.

Another method of collecting the saliva was worked out. This consisted in stimulating the flow of saliva from glands which had previously been in a resting state,[1] by irrigating the mouth with water or a weak acetic acid solution, followed by inhalations of strong acetic acid. In addition to these methods, tempting food was sometimes offered which apparently aided in the stimulation of the flow.

The saliva was caught in a clean beaker as it flowed from the mouth. Cattle will often retain the saliva in the mouth and sometimes swallow it, but by holding the head down and inserting the index finger into the mouth at the commissure of the lips, and pulling the commissure downward and outward the retained saliva will flow out. The mechanical stimulation of the clean finger will also help in some cases to induce secretion. Ten cubic centimeters could usually be obtained without much difficulty and quite often much more.

Blood serum which was used in the experiments was obtained by bleeding from the jugular vein. It was obtained by means of a hypodermic needle under aseptic precautions. Twenty to 30 cc. would be drawn into a sterilized flask, and at once placed upon ice. After several hours the serum was drawn off, centrifuged if necessary, and used.

METHODS OF STUDY

Clearing of the starch solution. To three test-tubes of equal size 5 cc. of a 1 per cent cooked Dakomin corn starch solution was added. To the first test tube, 1 cc. of clear or filtered ox saliva was added; to the second, 1 cc. of clear or filtered human saliva (obtained by washing the mouth and stimulating secretion by means of paraffin); and to the third, 1 cc. of distilled water. They were then incubated at room temperature or 40°C. depending upon the series to which they belonged. The three tubes were compared at certain intervals and the amount of clearing recorded.

Iodine tests. At intervals of a few minutes a drop from each of the above test tubes was added to a drop of Lugol's solution. The drops of Lugol's solution were placed upon a glass slab with a white background.

Reduction tests. A series of tubes was made in quadruplicate, similar to those used in the clearing tests. After being incubated for a certain length of time, each was tested with Fehling's solution. The Fehling's solution, alone and also with the cooked starch, was tested for reducing properties. The ox and human saliva was also tested with Fehling's solution.

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[1] In one case considerable saliva was being secreted at the time of making the collection, but this saliva showed as strong a diastatic power as any collected from glands which had previously been in a state of rest.

Serum tests. The experiments using blood serum in place of the saliva were carried out in a similar way.

RESULTS

The results here recorded are based upon the examination of eight different specimens obtained from six different mature bovine animals, of the dairy type. Slight digestion varying in degree was obtained in six examinations, and two were practically negative. The two negative samples were obtained from animals which later gave positive results.

Saliva of the ox is a clear viscid fluid, and when produced in large quantities, it will hang from the mouth in strings. It was always found to be alkaline to litmus.

Our experiments show that the ability of ox saliva to digest cooked starch varies in individuals and even in the same individual at different times. The action produced by 1 cc. of saliva is about equal, in some cases, to the action of four drops of blood serum, in others to about three drops, but when it becomes lower than this, it cannot be detected. Whether the saliva is obtained from glands which have been in an active state, or in a resting state, does not seem to make much difference.

Results of clearing the starch solution. Slight clearing of the starch solution containing 1 cc. of ox saliva could be seen in some cases at fifteen minutes, while in others it took one-half hour before any clearing could be detected. Complete clearing of the solution occurred in about one hour in the majority of cases. Those cases which were not clear after one to one and one-half hours never did become clear. There seemed to be very little, if any difference, in the rate of clearing between the samples incubated at room temperature and those at 40°C. Similar solutions of starch with human saliva and distilled water were used as controls in making the readings. In the case of human saliva the solution became clear in five to ten minutes, while the solution containing water only did not.

Results of iodine test. Although starch solution which contains ox saliva usually becomes clear within one hour, the blue color with iodine remains, nor does it tend to disappear after incubation has been carried on for a period extending over two hours. In the case of the controls, starch with human saliva showed no color with iodine after about ten minutes, while that of the starch and water continued to give the blue color throughout the incubation period.

Results of the reduction tests. Some cases gave a slight positive reduction at fifteen minutes while others did not, but whenever clearing was marked at fifteen minutes, a slight reduction occurred. If the starch solution was clear at one-half hour, a slight reduction would occur. In the majority of cases the longer the period of incubation, the greater the amount of reduction until the maximum was obtained, which occurred at one and one-half to two hours. Control reduction tests showed starch and human saliva to give a strong reduction after incubation for five minutes. The starch and distilled water tube was tested at the end of the incubation period and was always negative. Human saliva (1 cc.) alone does not reduce Fehling's solution, but 1 cc. of ox saliva will usually give a faint reduction.

Following is the detail of one experiment on the diastase of saliva of the ox.

Animal. Pure bred Guernsey cow. Ten cubic centimeters obtained by the usual methods. Reaction alkaline, glands in state of rest at time of stimulating secretion.

Tube A contains 5 cc. of 1 per cent starch and 1 cc. of ox saliva, made in quadruplicate and labelled A, A^1, A^2, A^3. Tube B contains 5 cc. of 1 per cent starch and 1 cc. of human saliva, made in quadruplicate and labelled B, B^1, B^2, B^3. Tube C contains 5 cc. of 1 per cent starch and 1 cc. of distilled water.

Clearing

NUMBER OF TUBE	TIME IN MINUTES					
	15	30	45	60	90	120
A's...	Slight	Slightly more	Slightly more	Clear		
B's...	Considerable	Clear	Clear	Clear		
C.....	No action	No action	No action	No action	No action	No action

Iodine

NUMBER OF TUBE	TIME IN MINUTES											
	5	10	15	20	25	30	35	40	45	60	90	120
A.....	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
B.....	Blue	No color	No color									
C.....	Blue	Blue				Blue			Blue			Blue

Reduction

NUMBER OF TUBE	TIME IN HOURS			
	½	1	2	2½
A's...	A. Slight reduction.	A^1. Slightly more	A^2. Slightly more	A^3. Same as A^2.
B's...	B. Much reduction.	B^1. Same.	B^2. Same	B^3. Same
C.....				No reduction.

Checks.

1. Fehling's solution plus boiling—negative.
2. Starch and Fehling's plus boiling—negative.
3. Human saliva plus Fehling's plus boiling—negative.
4. Ox saliva plus Fehling's plus boiling—faint trace.

These tests were made with equal amounts of material, carefully measured with a pipette and made in equal sized test tubes. Ten cubic centimeters of Fehling's solution was used in each case.

Details of experiment to show when reduction first occurs. Six tubes each containing 5 cc. of 1 per cent starch and 1 cc. of ox saliva

NUMBER OF TUBE	LENGTH OF INCUB. PERIOD	RESULTS OF REDUCTION TESTS
	minutes	
1	5	No reduction
2	10	No reduction
3	15	No reduction
4	20	Trace
5	25	Slight
6	30	Slight

RESULTS OF BLOOD SERUM EXPERIMENT

There is present in the blood serum of the ox an enzyme which behaves very similarly to the one found in the saliva. In the serum it is much more concentrated and three to four drops of blood serum exerts an action upon starch equal to 1 cc. of saliva. When three to four drops are used, the starch solution becomes clear in one-half to one hour. Reduction of Fehling's solution may occur after fifteen minutes of incubation, but is most pronounced after one to two hours. The blue color with iodine remains throughout.

Detail of series of experiments on the relative diastatic action of ox's serum. Digestion at 40° C.

SERUM SERIES	AMT. OF SERUM USED	AMT. OF STARCH USED	CLEARING						IODINE						REDUCTION				
			15	30	45	60	90	120	5	15	30	45	60	120	15	30	60	90	120
	drops	*cc.*																	
1	2	5	No action	No action	No action	Slight clearing	Slight clearing	Slight clearing	Bl	ue	at	all	tim	es	No Reduct.	No Reduct.	No Reduct.	No Reduct.	No Reduct.
2	3	5	No action	No action	No action	Slight clearing	Slight clearing	Slight clearing	Bl	ue	at	all	tim	es	No Reduct.	Trace	Considerable	Slight	Considerable
3	4	5	Slight clearing	Clearer	Clearer	Clear	Clear	Clear	Bl	ue	at	all	tim	es	Slight	Slight	Considerable	Considerable	Considerable
4	6	5	Quite clear	Clearer	Clear	Clear	Clear	Clear	Bl	ue	at	all	tim	es	Considerable	Considerable	Much	Much	Much
5	8	5	Quite clear	Clearer	Clear	Clear			Bl	ue	at	all	tim	es	Much	Much	Much	Much	Much
6	15	5	Quite clear	Clear	Clear	Clear			Bl	ue	at	all	tim	es					Much

1. Two drops of serum and Fehling's solution.—No reduction.
2. Three drops of serum and Fehling's solution—faint trace of reduction.
3. Four drops of serum and Fehling's solution—trace of reduction.
4. Six drops of serum and Fehling's solution—trace of reduction.
5. Eight drops of serum and Fehling's solution—trace of reduction.
6. Fehling's solution plus boiling—negative.
7. Fehling's solution plus starch, 5 cc. at 40° C. for two hours—negative.

THERMAL DEATH POINT

It was found in one series of experiments that heating the enzyme found in the serum to 63°C. for one minute will greatly inhibit the action of the enzyme, and heating for one minute at 65°C. will practically destroy its activity.

NUMBER OF TUBE	DEGREE OF HEAT FOR 1 MIN.	LENGTH OF INCUB.	CLEARING		REDUCTION
			½ hr.	1 hr.	
		hour			
1	No heat	1	Clear	Clear	Much
2	63°	1	Almost clear	Clear	Good reduction. not as much as in 1
3	65°	1	No action		Slight trace
4	75°	1	No action		Slight trace
5	84°	1	No action		Slight trace
6	90°	1	No action		Slight trace
7	Boil	1	No action		No reduction

Ten drops of serum used.
Ten drops, plus Fehling's—Slight trace.

DISCUSSION

This starch-splitting enzyme or enzymes found in the saliva of the ox acts much slower than the starch-splitting enzymes found in human saliva. It is present in very minute traces and in some samples of saliva cannot be demonstrated. This enzyme or enzymes is much more concentrated in the blood serum and is very likely carried into the saliva, along with other salivary constituents, which accounts for the small amount present in the saliva.

Owing to the nature of the food which this animal takes, one could naturally expect the serum to contain a relatively large amount of diastatic ferments, and it would only be natural for some of these ferments to be excreted in the saliva.

Carlson and Ryan (5) found a similar condition existing in the cat. They found that the saliva of the cat contains a starch splitting ferment. This ferment is not a specific product of the salivary glands but the diastase of the blood carried into the saliva along with other salivary constituents. They also found the starch splitting enzyme, or enzymes, to be very much more concentrated in the cats' serum than in the cats' saliva.

Because of the slowness of action of this ferment in the saliva of the ox and the small amount present, it is doubtful if it has any digestive value. It would seem that if nature intended the saliva of the ox should contain a starch splitting enzyme, it would be present in larger quantities and be more rapid in its action, and that it would resemble ptyalin of man in these respects.

SUMMARY

It is recognized that the number of observations is small for drawing definite conclusions, but that the uniformity of results points strongly to the conclusions drawn.

1. Saliva of the ox contains a starch splitting enzyme or enzymes, which varies in amount in individuals, and at different times in the same individual.
2. This enzyme or enzymes is not a specific constituent of the saliva but comes from the blood which contains similar enzymes in much higher concentration.
3. These enzymes have a thermal death point at about 65°C. when heated at this temperature for one minute.
4. This enzyme or enzymes is unimportant as far as its digestive ability is concerned.

I wish to express my great appreciation to Dr. C. J. V. Pettibone for his constant interest and advice.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS[1]

IV. The Effect of Thyroid Secretion on the Pressor Action of Adrenin

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The employment of organ extracts to determine the physiological effects of the specific secretions of endocrine glands has, in a number of instances, led to much confusion and contradiction. Schäfer (1) has recently emphasized the fact that "we are not justified in assuming that because we obtain evidence of the presence of active substances in such extracts that they are necessarily preformed in the organ and still less that they are passed out from it into the blood." The widely different effects produced on the circulation by intravenous injection of variously prepared thyroid extracts afford a striking example of the uncertainty of a method in which an impure product is used. Thus, whereas some investigators have observed a rise in blood pressure, others report a fall, while still a third group finds no change whatsoever. Similarly discordant observations are recorded with regard to changes in pulse rate. Unless an active principle in pure form is available, as is the case in the secretory product of the adrenal medulla, it would seem desirable, where possible, to study the physiological effects of an internal secretion by stimulating to activity the gland producing it.

The recent work of Cannon and Cattell (2), reported in a previous paper of this series, has placed the secretory innervation of the thyroid on a firm basis. These observers have employed as their criterion of secretory activity the electrical change giving rise to the current of action. They have demonstrated that an action current is produced in the thyroid gland by stimulation of the cervical sympathetic nerve or by injection of minute amounts of the sympatheticomimetic substance, adrenin (3). The gland secretes promptly on stimulation, i.e., an action current is obtained after a latent period of only a few seconds. No evidence of secretory activity is observed on stimulating the vagus nerves nor is there any electrical change after injection of the vagotropic drug, pilocarpine.

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[1] The earlier papers of this series were published in this Journal, 1916 (July), xli, pp. 39, 58 and 74.

As a result of these studies, two methods are at hand for inducing secretory activity in the thyroid gland. It is the object of the present communication to present evidence of the presence of thyroid secretion in the circulating blood after stimulation of the cervical sympathetic nerves or after injection of adrenin and to demonstrate that thyroid secretion increases the effectiveness of adrenin in raising arterial pressure. Its effects on blood pressure and pulse rate are also recorded.

PREVIOUS WORK ON THE EFFECT OF THYROID SECRETION ON THE PRESSOR ACTION OF ADRENIN

Having been led to believe from the work of certain Viennese investigators (4) that thyroid and adrenal glands mutually stimulate one another, Asher and Flack (5), in 1910, set out to determine whether during stimulation of the laryngeal nerves, which they regarded as the secretory nerves of the thyroid, a given dose of adrenin would produce a greater circulatory response than before. They demonstrated to their own satisfaction that the rise in blood pressure during stimulation was higher and of longer duration than during a previous control period and felt justified in concluding that (1) stimulation of the laryngeal nerves causes secretion of the thyroid gland; (2) thyroid secretion sensitizes sympathetic endings to the action of adrenin. These results were obtained fairly consistently in rabbits, but inconstantly in cats. Their tracings are far from convincing, since the differences in the height of the curves before and after stimulation amount to but a few millimeters, and are not more than might reasonably occur during variations in the depth of anesthesia or from respiratory fluctuations. Aside from this criticism, Cannon and Cattell (6) have shown that the laryngeal nerves, as branches of the vagi, are in no way directly concerned with secretory discharge in the thyroid gland.

In a brief preliminary report recently published, Oswald (7) records a series of observations on the effect of injection of iodthyreoglobulin on the circulation. It produces no alteration in blood pressure or pulse rate. But after intravenous injection of this substance, which Oswald calls the true secretory product of the thyroid, adrenin causes a rise in pressure which is higher and of longer duration than before.

The curve may, in fact, be twice as high and twice as long (no tracings are reproduced.) This effect is manifest, however, only after a short latent period and persists for a considerable time, having been demonstrated after the lapse of 1½ hours. It is Oswald's belief, therefore, that iodthyreoglobulin renders sympathetic endings more sensitive to the action of adrenin and he suggests that both latent period and long duration of effect are probably dependent on the colloidal nature of his product.

These studies are suggestive. But Asher and Flack, according to most recent evidence, did not stimulate the true secretory nerves of the thyroid and Oswald injected a glandular preparation the identity of which with thyroid secretion was an assumption.

METHODS OF THE PRESENT INVESTIGATION

Cats were used in all the experiments. The animals were prepared essentially according to the method described by Elliott (8) for use in his physiological quantitation of adrenin solutions. After preliminary ether anesthesia, a tracheal cannula was inserted. Care was taken not to injure the large vein running along the trachea, since it carries in large part the venous return from both thyroid glands. To destroy the brain, a perforation was made in the back of the orbit and a probe was passed through it into the spinal canal beyond the level of the medulla. Artificial respiration, maintained by a motor-driven, air-blast interrupter was now begun. The depth and rate of respiration must be carefully adjusted at the start if the animal is to be kept in good condition for a number of hours and these factors should not be altered during the course of an experiment. Both vagi were cut. The cervical sympathetic nerves were dissected free from the vagus sheaths for 2 or 3 cm., ligated low in the neck and severed distal to the ligatures. The depressor, when present as a separate nerve, was also cut. The probe was now again inserted through the orbit and the cord thoroughly destroyed to about mid-thorax, a level corresponding to the third or fourth thoracic segment. Usually in the course of twenty minutes or half an hour the blood pressure fell to a constant level between 25 and 50 mm. Hg. An electric heating pad placed beneath the animal permitted the maintenance of a normal or nearly normal temperature.

Medium sized or small cats (2 to 3kg.) were found more suitable than larger animals. Males were used almost entirely in order to avoid the variable conditions in the female genital organs. In cats pithed as described, the resulting rise in blood pressure after an injection of adrenin varies directly with the amount injected. Vasomotor and respiratory fluctuations are abolished. In the words of Elliott, "the circulatory system responds with the accuracy of a chemical balance to any dose of adrenin." In certain instances, despite repeated pithing, irregular and wavy curves were obtained; it was found best under these conditions to reject the animal and make a fresh preparation.

A cannula inserted into the femoral artery was connected with a mercury manometer, sodium carbonate being used as anticoagulant. Injections of adrenin were made through another cannula placed in the femoral vein on the opposite side. A slowly revolving kymograph was set so that the speed was uniform in all experiments.

The adrenin employed for injection was the commercial "Adrenalin Chloride" of Parke, Davis and Company, and was fresh. Immediately before each experiment it was diluted, usually 1:100,000, with distilled water, in which the adrenin remains without obvious chemical change for a considerable period. The injections were made from a syringe with 1 cc. barrel, graduated to tenths and fiftieths of a cubic centimeter. A metronome, beating at intervals of one second, permitted delivery of the dose uniformly in successive injections. Usually 0.2 or 0.3 cc. were injected at the rate of 0.02 cc. per second.

For stimulating the cervical sympathetic nerves a tetanizing current, rhythmically interrupted at the rate of 24 times a minute, was employed, the nerve being laid over a platinum electrode supported by a clamp. The current was of such strength that it could comfortably be borne on the tip of the tongue and always caused maximal pupillary dilatation when the side with intact eye was stimulated.

A time marker registering 30-second intervals served also as base line for zero blood pressure and as signal.

Any deviation from the usual technique will be described in its proper place.

PRESENTATION OF RESULTS

Effects of sympathetic stimulation. In all of the experiments the first two or three injections served as controls.[2]

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[2] Due probably to incomplete filling of cannula and vein with the adrenin solution at the start, the first curve was often smaller than those made a few minutes later (see, for example, fig. 2), and has at times therefore been disregarded in the presentation of control tracings.

In figure 1, curve *C* shows the blood pressure record during the of stimulation. The slight rise beginning and ending promptly the stimulus is not to be regarded as specific, since a similar t obtains with thyroids removed. Moreover, by ligating the large in the neck on the stimulated side, the rise can be almost en abolished, indicating its vasomotor origin. It is significant, in of the discordant statements mentioned earlier in the paper, that is no appreciable effect on blood pressure during sympathetic lation, since, as will be pointed out later, throughout this tin thyroid is pouring out its secretion.

The height of the adrenin rise immediately after stimulatio even 35 minutes later (see fig. 1, *D*, and fig. 2) is no higher tha obtained in the controls. It is only after a *latent period*, in this ins about 50 minutes, that the increased effectiveness of adrenin is ma The latent period may vary from about 40 to 60 minutes, but is a observed. The effect of the thyroid secretion, when once its acti evident in augmenting the circulatory response, increases in usually for several hours, and may persist for as long as 7 hour figs. 11 and 12.)

In Experiment XII (figs. 1 and 2) the maximum effect was obs some 100 minutes after stimulation, when the rise obtained was cent higher than in the control. Considerably greater increases occur. It is of interest that the ratio between the height of the or rise and that produced after stimulation represents precisely t crease in the efficacy of a given dose of adrenin. For example, if (1:100,000) causes before stimulationa rise of 25 mm. Hg., an duces two hours after stimulation a rise of 75 mm. Hg., injecti 0.1 cc. of adrenin will accurately reproduce (within 2 or 3 mm. the original curve at this later time.

There is a distinct variability in different animals in the magn of the effect resulting from stimulation. Several cats with "sn and a few lean, scrawny animals which had recently been shipp the laboratory in crates from a distance showed remarkably increase in pressor response.

Tracings taken on a more rapidly revolving drum have shown neither during nor after stimulation is there any significant alte in pulse rate. A considerable acceleration of heart rate accom each adrenin injection. A series of observations was recorded termine if, coincident with the augmented efficacy of adrenin in ing arterial pressure, there was also a greater pulse-rate increase height of the rise. Table 1 indicates that such an increase do occur, and serves also as an illustration of a typical protocol (abbreviated):

TABLE 1.

Effects of sympathetic stimulation

Experiment LIX. June 24. Small, female cat.

10.10 Ether. Struggled vigorously.

10.15–10.30 Tracheotomy. Pithed through medulla. Vagi cut. Sympathetics dissected and ligated.

10.35 Pithed to mid-thorax. On warming pad.

		RISE IN BLOOD PRESSURE (MM. HG.)	PULSE BEFORE INJECTION	PULSE AT HEIGHT OF RISE	TEMPERATURE
11.15	Injection No. 1. 0.3 cc. adrenin, 1: 100,000 at rate of 0.02 cc. per second.	34	153	225	37
11.23	No. 2	35	156	213	
11.28–11.33	Stimulation of left cervical sympathetic.				
11.45	No. 3	30	162	216	37
11.55	No. 4	34	165	213	
12.10	No. 5	42	165	216	
12.50	No. 9	50	171	222	37.5
12.57–1.02	Stimulation of right cervical sympathetic				
1.05	No. 10	55	171	225	
1.30	No. 12	61	171	213	
2.30	No. 15	70	162	214	
2.40	No. 16	70	165	212	37.5

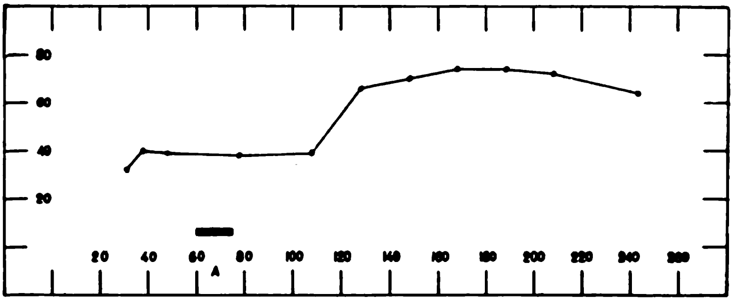

Fig. 2. Complete chart of Experiment XII. (See fig. 1.) Here, as in all other charts, ordinates represent blood pressure in mm. Hg., abscissae time in minutes. The dots (joined by solid lines) denote the height of the blood pressure rise resulting from injection of the specified amount of adrenin. At *A*, stimulation of the left cervical sympathetic nerve for 15 minutes.

Effect of injecting a large dose of adrenin. Adrenin mimics sympathetic impulses, and Cannon and Cattell, after its injection, have demonstrated an action current in the thyroid. Figures 3 and 4 (Experiment XVIII) picture the results after injecting 10 cc. of adrenin, 1:100,000. There is the usual latent period followed by gradual augmentation in pressor response until, 160 minutes after the injection, the rise produced is 82 mm. Hg. as compared to the control of 18 mm. Hg.—an increase in effectiveness of over 300 per cent! The removal of both thyroids at *B*, figure 4, did not prevent the effect from continuing for a number of hours, though as seen in figures 1, 2, 3 and 4 there is at times a tendency for the curve to diminish toward the end of the experiment.

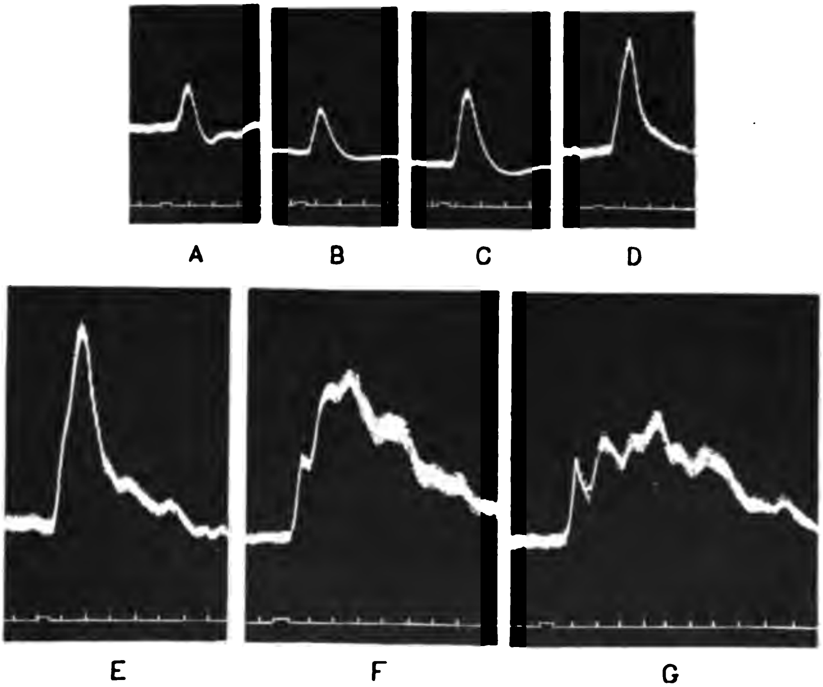

Fig. 3. Experiment XVIII. Effect of injecting a large dose of adrenin. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1:100,000. *A*, control. (13 minutes after *A*, injection of 10 cc. adrenin, 1:100,000, during a 10-minute period). *B*, 32 minutes after the injection. *C*, 60 minutes after the injection. *D*, 102 minutes after the injection. *E*, 162 minutes after the injection. *F*, 327 minutes after the injection.

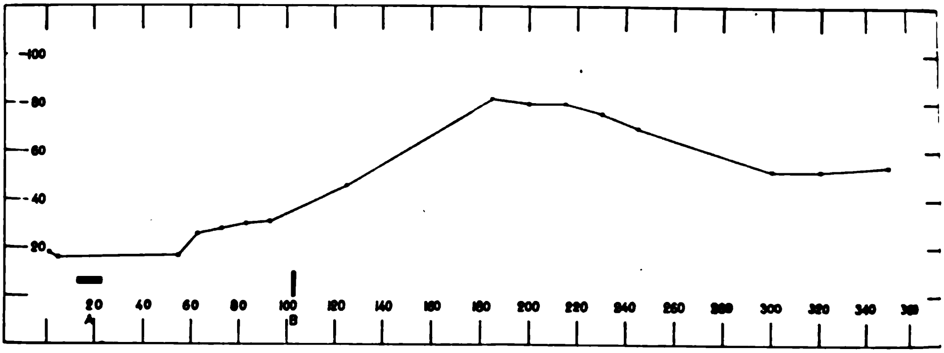

Fig. 4. Complete chart of Experiment XVIII., (See fig. 3.) At *A*, injection of 10 cc. adrenin, 1: 100,000, during a 10-minute period. At *B*, both thyroid glands removed.

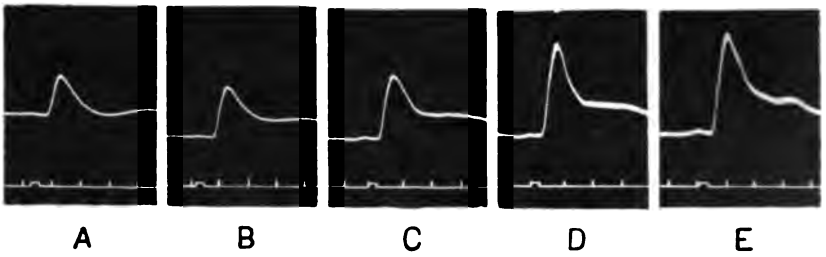

Fig. 5. Experiment XIII. Effect of repeated injections of minute doses of adrenin. Curves show rises in blood pressure after injection of 0.2 cc. adrenin, 1: 100,000. *A*, control. *B*, 35 minutes after *A*. *C*, 100 minutes after *A*. *D*, 120 minutes after *A*. *E*, 135 minutes after *A*.

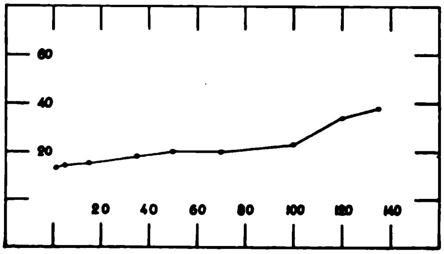

Fig. 6. Complete Chart of Experiment XIII. (See fig. 5.)

Effect of repeated injections of minute doses of adrenin. Repeated testing of the circulatory response requires the repeated injection of small doses of adrenin at intervals; and the electrical evidence of Cannon and Cattell indicates that even minute doses are sufficient to induce secretory activity in the thyroid. In Experiment XIII, (figs. 5 and 6) 9 injections of 0.2 cc. adrenin, 1:100,000, were made in the course of 135 minutes. Again there is seen the gradual gain in the height of the rise, from a control of 14 mm. Hg. to a response of 38 mm. Hg., an increase of over 160 per cent.

A few of the cats struggled vigorously while being placed in the holder and during the stage of primary anesthesia. They took the ether poorly. Their tail-hairs stood on end and the pupils became widely dilated. It was noted on beginning to take control records some 40 or 50 minutes later that there was a progressive increase in the height of the three curves, though taken at 5 or 10 minutes intervals. Emotion (9), ether (10) and asphyxia (11), all conducive to adrenal discharge, had apparently caused sufficient liberation of adrenin to stimulate the thyroid. It is probable that there was also direct nervous stimulation of the thyroid through impulses passing along the cervical sympathetic. The 40 or 50 minutes required to prepare the animal for the experiment had served as the necessary latent period and the thyroid secretion at once manifested its presence.

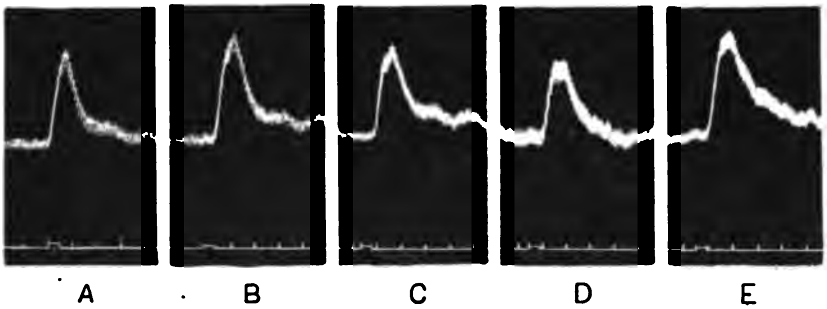

Fig. 7. Experiment LX. Failure of effect of cervical sympathetic stimulation after removal of both thyroid glands. Double thyroidectomy performed 1 hour and 45 minutes prior to first adrenin injection. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1: 100,000. *A*, control. (8 minutes after *A*, stimulation of left cervical sympathetic for 5 minutes, immediately followed by stimulation of the right cervical sympathetic for 5 minutes.) *B*, 2 minutes after stimulation. *C*, 31 minutes after stimulation. *D*, 86 minutes after stimulation. *E*, 131 minutes after stimulation.

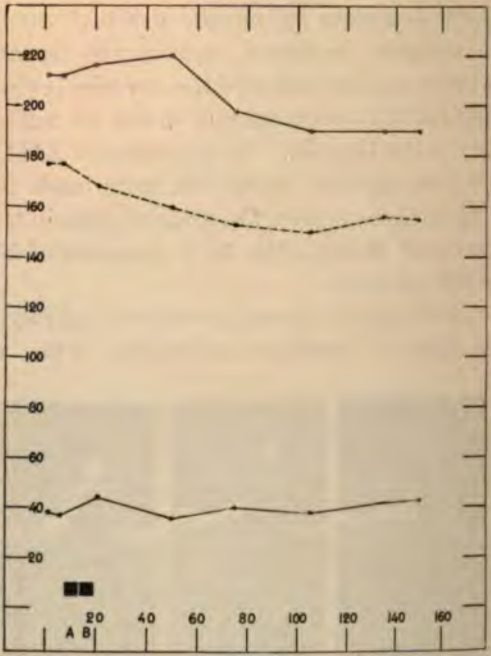

Fig. 8. Complete chart of Experiment LX. (See fig. 7.) Dots, joined by lines (lower curve), represent rises in blood pressure as in other charts. joined by interrupted lines (middle curve), represent pulse rate before inj of the standard dose of adrenin. Circles, joined by solid lines (upper c represent pulse rate at the height of the adrenin response. At *A*, stimu of the left cervical sympathetic for 5 minutes; at *B*, stimulation of the right cal sympathetic for 5 minutes.

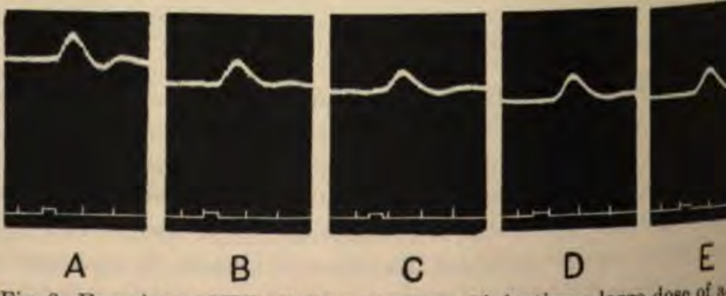

Fig. 9. Experiment XIX. Failure of effect of injecting a large dose of a after removal of both thyroid glands. Double thyroidectomy perfor hour and 10 minutes prior to first adrenin injection. Curves show rises in pressure after injection of 0.3 cc. adrenin, 1: 100,000. *A*, control. (20 m after *A*, injection of 5 cc. adrenin, 1: 100,000, during a 5-minute period.) minutes after the injection. *C*, 35 minutes after the injection. *D*, 60 m after the injection. *E*, 80 minutes after the injection (and 105 minutes aft

been reported by numerous observers (Krause, Fraenkel, Trendelenburg, Ott and Scott and others) that in clinical states of hyperthyroidism, as well as after injection into animals of thyroid extract, there is an increase in the adrenin content of the blood and the assumption has therefore been made that thyroid secretion acts as a direct stimulant to the suprarenal capsules causing them to yield adrenin to the blood in large quantities. It is suggestive also that in cats with experimental hyperthyroidism Cannon, Binger and Fitz (13) found the adrenal glands to be nearly three times their normal size.

It might, therefore, be inferred that the increased effect of adrenin in raising blood pressure after inducing secretion in the thyroid is due to a greater amount of circulating adrenin which would make the injected dose more efficacious. Against this explanation, to be sure, is the absence of a rise in pressure after stimulation, the invariable latent period, in spite of the fact that both thyroids and adrenals secrete promptly, and the fact that none of these conditions are consistent with a gradually increasing effect.

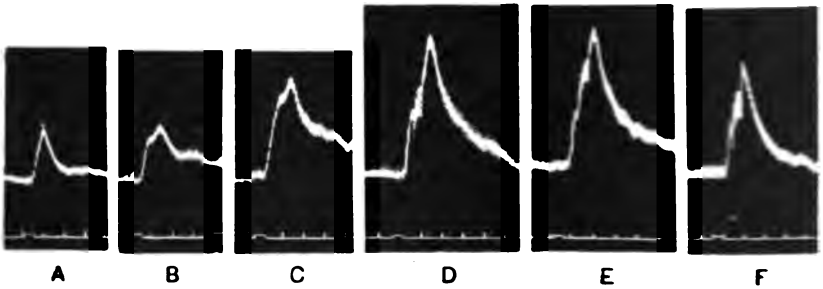

Fig. 11. Experiment XL. Effect of sympathetic stimulation followed by immediate removal of both thyroid glands. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1:100,000. *A*, control. (14 minutes after *A*, stimulation of left cervical sympathetic for 5 minutes, immediately followed by stimulation of the right cervical sympathetic for 5 minutes. Five minutes later, double thyroidectomy.) *B*, 35 minutes after stimulation. *C*, 125 minutes after stimulation. *D*, 210 minutes after stimulation. *E*, 360 minutes after stimulation. *F*, 465 minutes after stimulation.

The final proof is brought in Experiment XLIV (figs. 13 and 14) in which a double adrenalectomy through a median incision[3] was

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[3] It was found feasible to pith only brain and medulla in animals on which laparotomy was to be performed, since this operation on cats pithed to mid-thorax invariably caused a dangerous or fatal fall in pressure. Failure to destroy

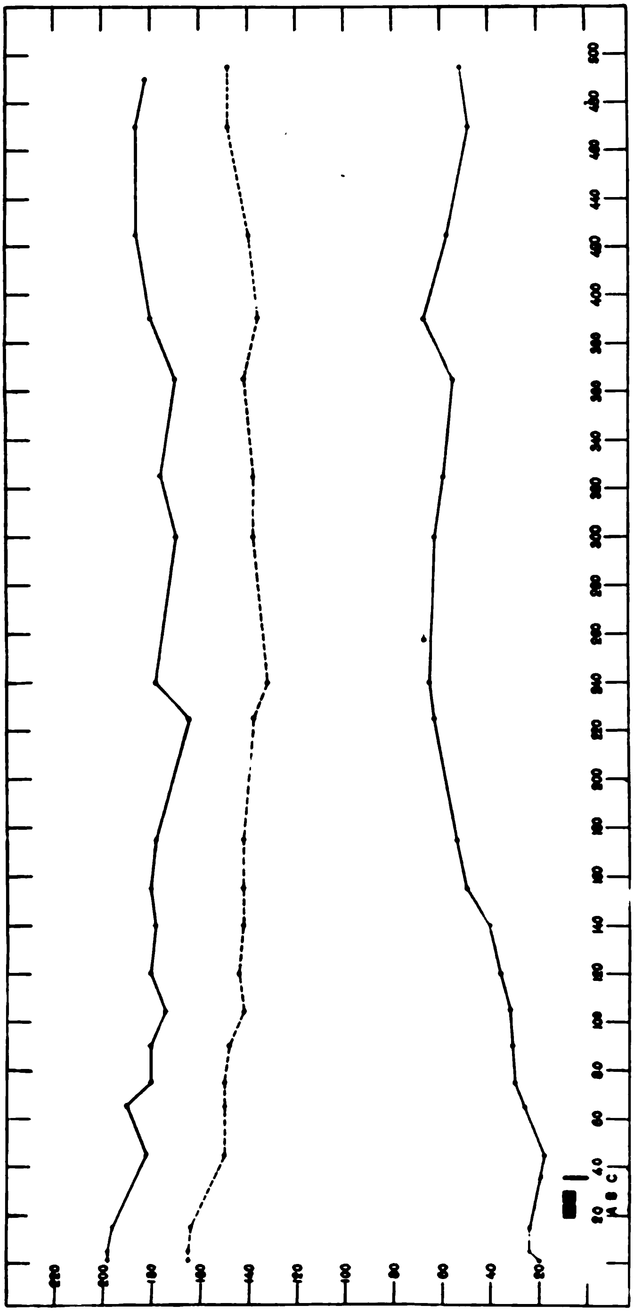

Fig. 12. Complete chart of Experiment XL. (See fig. 11.) Dots, joined by solid lines (lower curve), represent rises in blood pressure, as in other charts. Dots, joined by interrupted lines (middle curve), represent pulse rate before injection of the standard dose of adrenin. Circles, joined by solid lines (upper curve), represent pulse rate at the height of the adrenin response. At *A*, stimulation of the left cervical sympathetic for 5 minutes; at *B*, stimulation of the right cervical sympathetic for 5 minutes. At *C*, double thyroidectomy.

performed 50 minutes prior to the first adrenin injection. The absence of the ordinary latent period is not surprising, since Crowe and Wislocki (14) have pointed out that any operative procedure on either adrenal liberates sufficient adrenin to produce glycosuria and this amount would undoubtedly suffice to initiate secretory activity in the thyroid. The increase in the size of successive curves prevails as in the unoperated animals.

In other similar experiments in which the adrenal veins were more quickly ligated on entering the abdomen, the usual latent period was observed:

Effects of injecting the crystalline, iodin-containing compound isolated from the thyroid by Kendall (15). Through the kindness of Dr. E. C. Kendall of the Mayo Clinic, it has been possible to experiment with this substance, which, in minute doses has produced strikingly beneficial results in cretins and myxedematous individuals (16). A solution containing 0.3 mg. per cubic centimeter of the crystalline compound and having an iodin content of 0.184 mg. per cubic centimeter (60 per cent) was employed. The dosage used was approximately 1 mg. of the crystals per kg. of body weight.

Intravenous injection into previously thyroidectomized cats caused no appreciable alteration in blood pressure or pulse rate but did produce an increase in the effectiveness of adrenin as a pressor agent, as in the stimulation experiments. (See table 2.) Two points are worthy of special note; first, there was no marked latent period after injection of Kendall's compound, the effect being demonstrable a few minutes after its introduction into the circulation; second, the duration of the effect was relatively shorter than in the experiments in which the thyroid gland was stimulated in the animal body.[4]

Critical discussion. Kepinow (17) and others have demonstrated that after injection of extracts of pituitary, there is an increase in the

subsidiary vasomotor centers probably accounts for the greater variations in the basal blood-pressure levels in these experiments. Such variations are of no significance, however, since Asher (Hering-Festschrift, 1910, Hager, Bonn) has shown that the effect of vasomotor stimulation is the same whether the initial blood pressure be high or low.

[4] As has been mentioned earlier in this paper, Oswald has observed both latent period and long duration of effect after injecting iodthyreoglobulin and has explained them both on the basis of the colloidal nature of his product. It is conceivable that this explanation of these phenomena holds good also with regard to the secretion liberated by the gland in the body. The more rapid manifestation of activity and shorter effective period of Kendall's preparation may possibly be dependent on its crystalline character.

TABLE 2.

Effects after injection of Kendall's crystalline, iodin-containing thyroid compound

Experiment LVIII. June 23. Small, female cat. Weight, 1.9 kg.

9.30–9.42 Ether. Thyroidectomy (complete). Pithed through medulla. Both vago-sympathetic trunks cut. Pithed to mid-thorax. On warming pad.

		RISE IN BLOOD PRESSURE (MM. HG.)	PULSE BEFORE INJECTION	PULSE AT HEIGHT OF RISE
10.20	Injection No. 1. 0.3 cc. adrenin, 1:100,000, at rate of 0.02 cc. per second.	48	144	201
10.25	No. 2	51	147	207
10.35	No. 3	52	148	207
10.52–10.55	5 cc. compound, intravenously			
11.00	No. 5	62	156	198
11.25	No. 7	74	159	198
12.20	No. 11	72	156	195
12.30–12.33	5 cc. compound, intravenously			
12.35	No. 12	96	153	198
12.50	No. 13	92	162	207
2.20	No. 16	54	164	204
3.05	No. 18	45	141	184

power of adrenin to raise arterial pressure. According to Weed, Cushing and Jacobson (18) the pituitary gland secretes actively on stimulation of the superior cervical sympathetic ganglion. Pituitary secretion, however, cannot play any rôle in modifying the results of the experiments herein described. For the upper central nervous system and its circulation are so thoroughly destroyed by pithing that there can be no appreciable absorption from the subarachnoid space. And incontrovertible is the fact that with the thyroid glands removed, though other conditions remain unchanged, the usual effect is absent.

Börner has pointed out that in rabbits, pituitary extract slows heart rate and causes diminished cardiac systole, and attributes the increased effectiveness of adrenin, after pituitrin injection, to its greater concentration in the blood as a result of mechanical slowing of the circulation. A similar criticism is not applicable to the present work. For, (1) at the time of increase in pressor response there is no slowing of heart rate; (see fig. 12 and table 1.); (2) there is no progressive lengthening of latent period between adrenin injection and circulatory

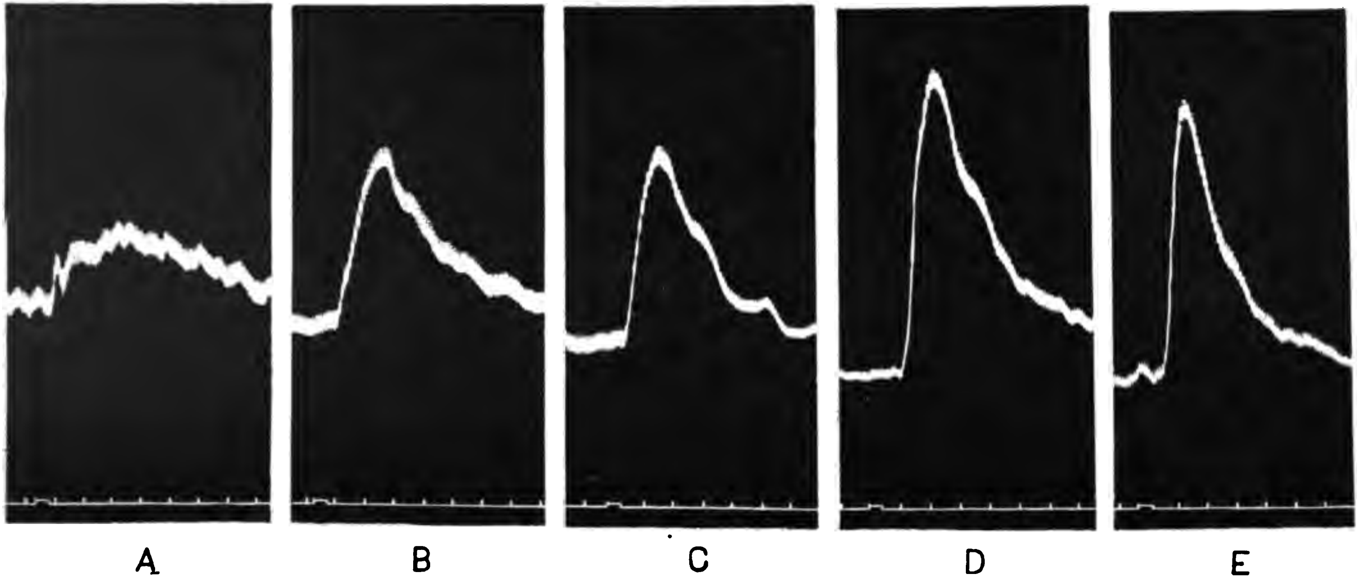

Fig. 13. Effect of cervical sympathetic stimulation after removal of the adrenal glands. Both adrenal glands removed through median incision 50 minutes prior to first adrenin injection. Curves show rises in blood pressure after injection of 0.3 cc. adrenin, 1:100,000 *A*, control. *B*, 55 minutes after A. *C*, 180 minutes after *A*. (5 minutes after *C*, stimulation of left cervical sympathetic for 5 minutes, immediately followed by stimulation of the right cervical sympathetic for 5 minutes.) *D*, 325 minutes after *A*, and 130 minutes after stimulation. *E*, 400 minutes after *A*, and 205 minutes after stimulation.

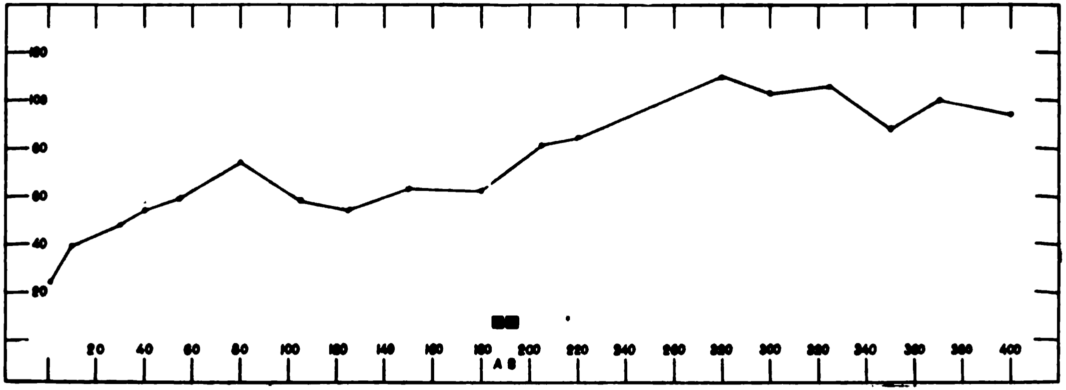

Fig. 14. Complete chart of Experiment XLIV. (See fig. 13.) At *A*, stimulation of left cervical sympathetic for 5 minutes. At *B*, stimulation of right cervical sympathetic for 5 minutes.

response as would occur if the circulation were slowed; (3) pulse pressure tends often to increase rather than diminish; (4) in a number of experiments the increase in the height of the curves has been followed by a subsequent diminution in their size (see figs. 1, 2, 3, 4, 11 and 12); and finally, (5) with thyroids removed, and therefore no increase in adrenin effectiveness, there may be a slowing of pulse rate during the course of the experiment (see fig. 8).

It is clear therefore, that the greater adrenin response after thyroid stimulation is due to a sensitization of the sympathetic structures acted on by adrenin in raising blood pressure. The action of thyroid secretion on sympathetic endings is a selective one, for there is no concomitant increase in excitability of the augmentors of the heart.

SUMMARY

1. In cats, after stimulation of the cervical sympathetic in the neck, there can be demonstrated an increase of the effectiveness of adrenin in raising arterial pressure. This increase may be as much as 200 to 300 per cent.
2. Injection of adrenin, even in minute amounts, produces a similar effect.
3. This effect is manifest only after a latent period, which may vary from about 40 to 60 minutes. There is then a progressive increase in the height of the curves, the maximum being reached in from 2 to 3 hours after stimulation. The effect is of considerable duration, having been observed for as long as 7 hours.
4. When the thyroid glands have been previously removed, cervical sympathetic stimulation or adrenin injection does not produce an increase in the pressor response to adrenin. It is therefore justifiable to conclude that (a) stimulation of the cervical sympathetic or adrenin injection induces secretory activity in the thyroid gland, (thus confirming the observations of Cannon and Cattell); and (b) thyroid secretion renders more excitable the sympathetic structures acted on by adrenin in raising arterial pressure.
5. Stimulation of the cervical sympathetic causes the thyroid to secrete promptly, since thyroidectomy immediately after stimulation is followed by a progressive augmentation in pressor response similar to that observed when the glands are left in the animal throughout the course of the experiment.

6. Intravenous injection into thyroidectomized cats of a solution of the crystalline, iodin-containing compound isolated from the thyroid by Kendall, is followed by an increase in the efficacy of adrenin as a pressor agent. The effect is demonstrable almost immediately after injection and is of relatively shorter duration than in the stimulation experiments. This substance causes no appreciable alteration in blood pressure or pulse rate.

7. Neither during nor after stimulation of the thyroid gland through its secretory nerves is there any significant change in blood pressure or pulse rate.

8. After thyroid stimulation, even at the time when adrenin is more effective as a pressor agent, there is no increase in the augmentation of pulse rate produced by adrenin injection. This indicates a selective action of thyroid secretion in sensitizing sympathetic tissues to the action of adrenin, since the increase in vascular response is not associated with greater excitability of the augmentors of the heart.

9. In cats whose adrenal glands have been previously removed, there can be demonstrated, after thyroid stimulation, an increase in pressor response similar to that seen in unoperated animals. Though there is some evidence in the literature indicating that thyroid secretion acts as a secretory stimulant to the adrenals, the increased effectiveness of adrenin as a pressor agent after thyroid stimulation is not dependent on a greater amount of circulating adrenin.

It is a pleasure to express my cordial thanks to Dr. W. B. Cannon for his helpful interest and advice.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

I. Volume Changes and Venous Discharge in the Limb

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In several papers from this laboratory the relation of various endocrine glands and particularly the suprarenals to the vasomotor system has been considered. In these studies the vasomotor apparatus was considered as a whole. The fact, however, that various components of the vasomotor system react differently to adrenin makes it desirable to investigate further the effects of that substance on vascular conditions throughout the body. This is in effect to determine the influence of adrenin on the distribution of the blood. Such studies are needed in case of various glands on account of their possible broad significance in endocrine biology. Theoretically any hormone might exercise a profound influence upon body functions by shifting the circulation in favor of one organ at the expense of another. This possibility has received little attention at the hands of investigators.

So far as we are aware the only investigators who have studied the specific problem of the relation of the suprarenals to the distribution of the blood are Falta and Priestly (1). Their method was to administer to an animal subcutaneously a large dose of adrenin (1 mgm. per kilo) and then from one to several hours later, when the animal was supposedly under the influence of the drug, to note the condition of various organs. This was done by opening the body, quickly clamping off the organs and observing the degree of anemia or hyperemia present. It was reported that the adrenin injections resulted in an accumulation of blood in the lungs, brain, liver and kidneys, while the skin, mucous membranes, spleen and muscles were pale. The conclusion was reached that the function of the suprarenals is so to regulate the blood vessel caliber throughout the body that an adequate circulation to the vital tissues can be maintained with a minimal cardiac effort.

This work is open to various objections. Whether subcutaneous injections of adrenin at all closely simulate adrenal discharge is doubtful. Kleiner and Meltzer (2) have found that so far as glycosuric effects at any rate are concerned the results of introducing adrenin into a vein and under the skin are notably different. Also mere inspection of the organs is but a rough way of determining their blood content and might well fail to disclose significant conditions. That the method was inadequate is indicated by the failure of the reported results to agree in some particulars with more adequate plethysmograph studies. For example there is no doubt that the liver and kidneys contract under the influence of adrenin although they were reported by Falta and Priestly as hyperemic.

The best known and most complete investigation of the effects of adrenin on the blood content of different organs is the first, made by Oliver and Schaefer (3) in 1894–1895. The reports of this work contain most of the pertinent data that have ever been recorded on the subjects treated. The work still needs supplementing, however, in certain respects. For example no determinations of changes of gut volume were made. Also their method of administering the extracts used was ordinarily by short lasting injections whereas normal adrenal discharge supposedly persists through a longer period of time. This persistence might permit secondary adjustments to occur and significantly alter the reaction picture. The possible effects of variations in dosage also failed to receive much attention, the supposition at that time being that such variations produce merely quantitative differences in the reactions. But investigations of more recent years have shown that the effects of adrenin may be diametrically different depending upon the amount used. For example the pulmonary vessels are dilated by small doses but constricted by large (4). In the respects mentioned the subject is still open to investigation.

The first studies of the effects of adrenin on limb volumes were reported in Oliver and Schaefer's paper just mentioned. It was found that the limb usually contracted. (In the literature they have been misquoted on this point.) In some of their experiments, however, an expansion of the limb was noted. These results have been confirmed by Vincent (5). It was found that the expansion was particularly likely to occur toward the end of an experiment. It was regarded as "passive." In certain instances one limb expanded while its fellow contracted. This inconstancy was ascribed to a struggle between active constriction of the arterioles and dilatation of the larger vessels within the plethysmograph.

Several investigators have recorded observations on the effects of adrenin on the outflow from perfused limbs. In the use of the Läwen-Trendelenburg perfusion method of assaying adrenin abundant proof has been secured that this substance in all dilutions causes vasoconstriction in the legs of the frog (6). Similar results have been reported by Brodie and Dixon (7), Embden and v. Fürth (8) and by Biedl (9).

Gerhardt (10) dissected out the vessels of a bit of living muscle and studied them under a microscope. When adrenin was injected he was not able to detect changes in the caliber of either the arterioles or the venules but concluded that the arterioles as a matter of fact must have constricted for otherwise the veins would have shown a dilatation as a result of the increased arterial pressure that ensued.

The foregoing observations have been interpreted as indicating that adrenin causes vasoconstriction in skeletal muscle. But the part played by the skin in such experiments has not received adequate consideration. That adrenin causes vasoconstriction in the cutaneous vessels has been reported by Velich (11), Baum (12), Elliott (13) and Vincent (14), From the study of the limb as a whole, therefore, without knowing the extent to which the skin participated in the reaction no conclusion is justified regarding the reaction in the muscle.

Hartman (15) has recently investigated the relative effects of adrenin in the "peripheral" and the splanchnic circulations. The superior and inferior mesenteric arteries, the coeliac axis and sometimes the renal arteries were tied off. Under those conditions it was noted that a dose of adrenin that had previously given a pressor reaction now gave purely or predominantly a fall of pressure. In other experiments the carotid and subclavian arteries and the abdominal aorta above the iliac were tied off. The circulation was thereby restricted largely to the trunk and thoracic viscera and the splanchnic region. Under such conditions the circulation was regarded as predominantly splanchnic. (That this was so follows from the work of Burton-Opitz (16) who found that the liver and kidneys receive a large proportion of the blood passing through the aorta.) Hartman found that when the peripheral circulation was so excluded the dosage of adrenin that normally gave predominantly a fall of pressure now gave a rise. In four cases the two types of experiment were alternated in the same animal. In each instance clamping the peripheral arteries transformed a depressor to a pressor reaction. The results of clamping the splanchnic vessels were less consistent but in general left unchanged or augmented the depressor effect. From these observations it would appear that a discharge of adrenin into the blood stream would result in a simultaneous constriction in the splanchnic and dilatation in the peripheral circulation. Since in these experiments the "peripheral circulation" included a large area of skin the vessels of which react by constriction it follows that the vessels of the muscles must have dilated. No experiments were made to determine to what extent the skin and muscle actually did participate in the reactions.

That a specific relationship exists between muscular efficiency and circulating adrenin has been asserted by several investigators. Oliver and Schaefer (17) showed in 1894 that suprarenal extracts increase the activity of skeletal muscle both in mammals and frogs. Dessy and Grandis (18), Panella (19) and Joteyko (20) have reported that adrenin improves the activity of amphibian muscle. According to Joteyko the effect is due not to stimulation of the muscle but to a change in the sarcoplasm that renders it more sensitive to nervous impulses. In amphibian muscle the effect is very slight.

In mammals the beneficial influence of adrenin is more marked and more easily demonstrated. Cannon and Nice (21) have shown that injections of the drug or stimulation of the suprarenal glands through their nerves improves the activity of stimulated muscle, sometimes 100 per cent. This favorable effect was noted even when the blood pressure was mechanically prevented from rising. Gruber (22) has found that adrenin greatly hastens the recovery of a muscle from fatigue. This result he concluded was not due to any influence on the vasomotor system whereby the circulation of the muscle was increased. No direct studies were made, however of the effects of adrenin on the circulation of the muscle under the conditions of his experiments.

As to how the beneficial influence of adrenin on muscle is brought about several possibilities present themselves. Among these are increased mobilization of dextrose, a direct influence of the adrenin on muscular metabolism and changes in the distribution of the blood favorable to the muscle. It was thought that further investigation might throw some light on this problem.

In this and subsequent papers will be reported the results of a series of studies of the effects of adrenin on the volume of various organs and on the venous outflow from them. The first structure to be considered is the limb or, more specifically, muscle and skin.

Methods. In all cases dogs were used as experimental animals. They were anesthetized with ether or ether and morphine.

The plethysmograph used for the leg was of the familiar Mosso type.

It was simply a glass cylinder of appropriate diameter closed at one end by a paraffined cork stopper through which passed an outlet tube. The other end was adapted to the leg by a thin rubber cuff. Leakage under the cuff was prevented by a liberal use of vaseline. Air transmission was employed. The outlet tube was connected to a recorder by rubber tubing in which was interpolated a glass T-tube. Through this the system was put under a positive pressure amounting to 4 or 5 cm. of water.

After considerable experimentation with different types of recorders that described below was improvised and adopted for routine use. It consists essentially of a water manometer and float and a counterpoised "heart lever" to which the movements of the float are transmitted. In the form first used the transmission was by means of an aluminium wire as shown in figure 1. Later a thread was substituted for the wire and found to be more satisfactory. The thread is attached to the float by means of a bit of colophonium wax. For a float an empty adrenalin ampoule serves admirably. Experience showed that the best results are secured when the bore of the manometer is only slightly greater than the outside diameter of the float. The instrument proved to have several points of superiority. As compared to a water manometer in which the thrust is transmitted directly to the writing point this has two advantages: The record can be written at any magnification

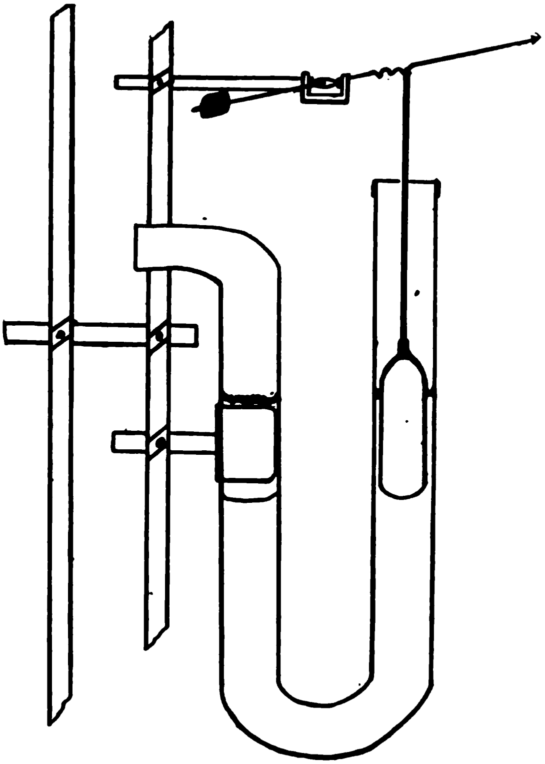

Fig. 1. Float recorder. (Diagrammatic; writing lever shown at reduced magnification.)

desired simply by adjusting the attachment of the transmission thread along the arm of the writing lever; also, the lateral extension of the writing lever provides considerable latitude for adjustment of the apparatus to the drum whereas when the direct thrust is transmitted as in the familiar mercury manometer it is difficult to arrange for two or more records in the same ordinate. As compared with a piston recorder of equal delicacy this has the advantage of requiring no lubrication. Also a bit of dust or distortion of the transmission from accurate alignment does not bring the apparatus to a standstill. The small cost, simplicity and ease of securing the apparatus are other obvious advantages.

Injections or infusions were made into a femoral vein through a cannula. The cannula was connected by a rubber tube with a reservoir of isosmotic saline solution. The tube was closed by a clip near the cannula. The drug was introduced into the tube and flushed instantaneously into the vein by opening the clip or it was infused more slowly from a burette through a hypodermic needle introduced through the rubber tube into the venous cannula.

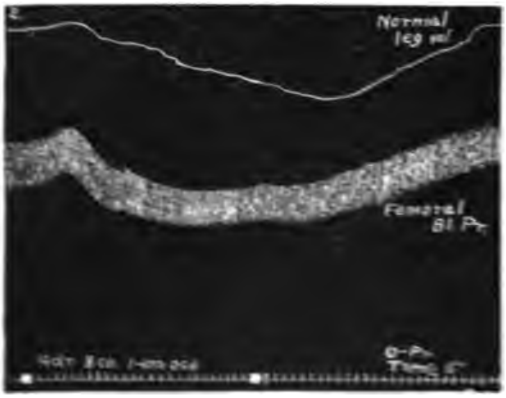

Fig. 2. Normal leg contracting under the influence of adrenin, depressor infusion. (Reduced to ½.)

As an index to the general efficiency of the dosages of adrenin employed arterial blood pressure was always recorded. In most cases the pressure was measured with a mercury manometer damped to exclude the pulse oscillations. These were recorded by means of a small tambour connecting with a side branch of the tube leading to the artery. This combined method, as pointed out by Sollman and Pilcher, has advantages over the use of either manometer or tambour alone.

Effects of adrenin on the intact limb. The effects of adrenin on the volume of the intact limb were first investigated. Varying dosages were used from those which gave a fall of pressure to those causing a marked rise. The duration of administration varied from about one second to ten minutes. Blood pressure from the femoral artery was recorded by the method previously described. Ordinarily the leg used in the plethysmograph was a forelimb but a hind limb also was occasionally employed. Dogs of about 14 kilograms weight were selected. The legs of these could be included in the plethysmograph up to the lower end of the humerus or femur.

Figure 2 shows the effect of a relatively small dose of adrenin administered by infusion. Eight cubic centimeters of 1–100,000 solution was introduced during a period of one hundred and thirty seconds. The leg volume at first increased slightly, the expansion lasting about thirty seconds, coinciding with a brief rise of arterial pressure. Then as blood pressure fell the volume decreased. The lowest point was reached, however, eighty-five seconds after blood pressure started to rise or thirty-five seconds after the infusion was discontinued. The blood pressure and leg volume reached their normal at the same time. This graph standing alone would probably be taken to indicate that the volume changes in the limb were passive. In many cases, however, such an interpretation is not possible.

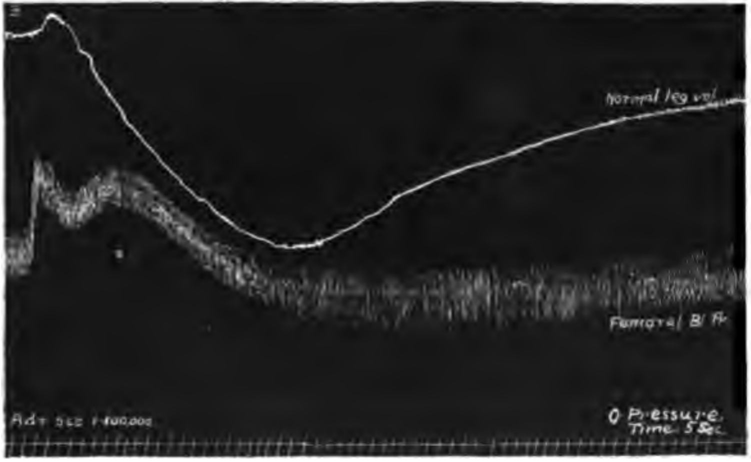

Fig. 3. Normal leg contracting under the influence of adrenin, pressor injection. (Reduced to ½.)

Figure 3 for instance shows a case in which an injection of adrenin caused a marked contraction of the leg which was clearly not a passive effect since the greater part of the contraction occurred while the blood pressure was above normal.

Figure 4 shows one of the cases that were occasionally seen in which an expansion instead of a contraction resulted from adrenin. The case is striking in that only a small instantaneous injection was used. The expansion in this case again is obviously not a passive effect; the limb did not begin to increase in volume until the pressor reaction was passed and it continued for some time after the pressure had reached essentially its normal level. In the light of later experience it is suspected that this was an unusually muscular dog but at the time the record was made the significance of that condition was not realized and no note on the point was made. Oliver and Schaefer reported that dilatation of the limb is most likely to occur toward the end of an experiment. In the case shown in figure 4 the experiment was just beginning.

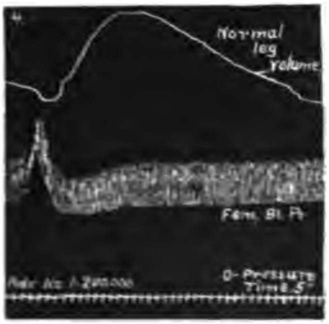

Fig. 4. Normal leg expanding under the influence of adrenin. (Reduced to ½.)

The results of this series of experiments as a whole were not unlike those of Oliver and Schaefer. In all 156 experiments were made in twenty-five different dogs. Seventeen dogs showed no other effect than contraction at any stage of the experiment or with any dosage. One showed expansion of the limb only while seven showed sometimes one and sometimes the other depending upon the dosage or a change in the condition of the animal during the course of the experiment. The conditions that could bring about a reversal in the volume reactions were not fully worked out but it was noted that hydremic plethora is one of them.

In most instances the effects of adrenin in the limb could not be interpreted as passive. There was little parallelism between the blood pressure and limb volume reactions. One experiment was particularly instructive in this respect. The dog was given a slow adrenin infusion lasting ten minutes. This resulted in a brief elevation of pressure followed by a return to normal and persistence at the normal level or slightly below normal during the whole of the infusion period. Both leg and spleen volumes were recorded. In both a sharply marked contraction occurred which lasted throughout the infusion period and promptly ceased immediately after the infusion was stopped.

One passive phenomenon only was noted with any degree of regularity; the leg volume usually was increased for a short time during the first pressor stage of the vasomotor reaction. This expansion according to our records is to be interpreted as due to the first effect of the adrenin in the heart. After it enters the aorta the drug is at once carried through the coronary circulation and stimulates the muscle augmenting the cardiac output while the peripheral resistance remains unaffected. This produces a brief initial increase in blood pressure which in turn causes a passive expansion of various organs at the expense of the great veins. In this expansion the limbs share. Then as the drug reaches the periphery the local effects come into play and give the volume curve its characteristic alterations. This feature is illustrated in figures 2 and 3. (In figure 3 the volume recorder was writing slightly in advance of the blood pressure manometer.)

Effects of adrenin in the skinned limb. The next step in the investigation was to determine whether and if so to what extent the limb contraction was due to vasoconstriction in the muscle. The components of the limb that have chiefly to be considered in such determination are the bone, muscle and skin. As to the bone its fixed volume precludes its taking any part, directly, at least, in the contractions or expansions of the limb. To determine the relative parts played by the muscle and skin the obvious method was followed of recording first the volume changes with the limb intact and then removing the skin and repeating the experiment.

The skin was incised all around just above the lower end of the humerus. It was then removed by traction aided by the occasional use of a scalpel. At the carpal articulation the paw was amputated. Ordinarily but few vessels had to be ligated. Oozing from the end of the stump was prevented by a tourniquet applied as a figure 8, the strands crossing between the ulna and radius. Despite the severity of the procedure the dogs usually showed little or no evidence of shock.

The results of removing the skin were striking and, on the whole, consistent. Under all conditions of dosage, duration of administration and resultant effects on blood pressure adrenin caused expansion of the skinned leg. One dog only offered an exception to this rule and that only with large doses. In all in this series one hundred and thirty-five injections or infusions were made in eighteen dogs. In some instances the leg was skinned after the reaction had been determined with it in its normal state. In other cases the experiment was begun with the skin removed. The experiment was also varied by recording simultaneously the volume changes in two legs, one being skinned and the other normal. The results were the same whatever method was used. Figure 5 illustrates a typical case. The first graph was taken with the leg in a normal condition. The skin was then removed and the adrenin injection repeated. The volume reaction was reversed by removing the skin. The animal in this case showed more shock than usual but a similar reversal occurred when the operation resulted in no apparent shock or when the changes in a normal and a skinned leg were recorded simultaneously. In different experiments a persistance of the limb expansion after the blood pressure had returned to normal indicates that the dilatation was not a passive process.

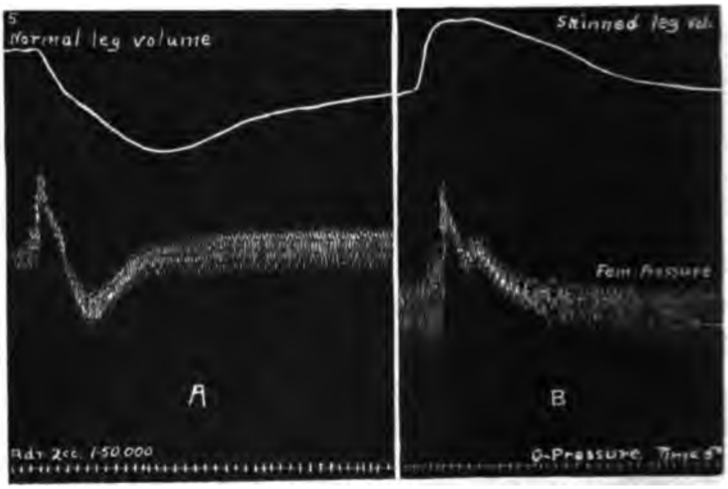

Fig. 5. *A*, Normal leg contracting; *B*, same leg after removal of the skin expanding under the influence of adrenin. (Reduced to ½.)

The objection might be offered that the trauma incident to removing the skin had induced a local vasomotor paresis which would account for the expansions observed. This objection could probably be met by removing the skin from the distal part of the leg leaving a considerable part of its circulation intact, taking a record with this skin wrapped about the limb and then repeating the injection with the skin left outside the plethysmograph. Indeed such an experiment was planned. But the results obtained in the experiments next to be described rendered this superfluous.

Throughout this series it was observed that removal of the skin from the leg caused it to expand under the influence of adrenin whereas the normal limb ordinarily contracts. The contraction therefore would seem to be due to vasoconstriction in the skin while the expansion in the skinned leg indicates that adrenin causes vasodilatation in the muscle. One's first impression is likely to be that the relative mass of the skin as compared with that of the muscle of the limb is too slight to permit vasoconstriction of the cutaneous vessels to overcompensate for vasodilatation in the muscles. As a matter of fact however, in the types of dogs used in these experiments the mass of the muscles in the lower segments of the legs was relatively small as compared with that of either the bone or skin. It is quite probable that with short legged unusually muscular dogs the vasodilatation would so predominate as to cause an expansion of the limb. A similar result would probably be obtained if it were feasible so to adjust the plethysmograph as to include the muscles of the proximal segment of the limb.

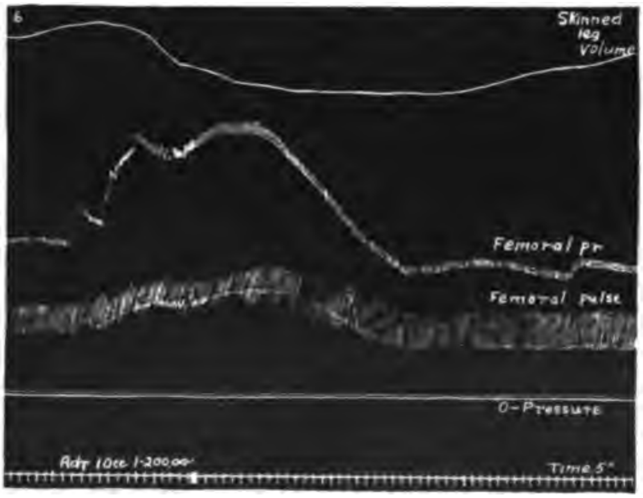

Fig. 6. Skinned leg contracting under the influence of adrenin, pressor infusion. (Reduced to ½.)

In case of one dog an exception to the ordinary effect was noted. This is illustrated in figure 6. Ten cubic centimeters of adrenin 1–200,000 was introduced during a period of eighty seconds. During the first half minute the volume of the skinned leg increased. Then a decrease occurred which persisted for some time after the blood pressure had returned to normal. The cause of the contraction in the absence of the skin was not determined. Possibly it might be ascribed to vasoconstriction in the bone such as Drinker and Drinker (23) have recently observed in perfusion experiments, the constriction holding back sufficient blood from the peripheral veins to cause a decrease in the total mass within the plethysmograph.

Effects of adrenin on venous outflow. Earlier in the paper mention was made of the work of different observers who found that adrenin perfused through the limbs decreased the rate of outflow. This was noted by Biedl (9) in the dog, the animal used in our experiments. Experiments on isolated limbs, however, tell little as to what would be the effect of an adrenin discharge in an intact animal. The effects of adrenin on the blood flow in the limb under normal conditions would depend upon the resulting arterial and venous pressures as well as upon local effects in the tissues involved. Furthermore, perfusion experiments made on the limb as a whole do not determine to what extent the changes of outflow depend upon effects in the skin, muscle or bone. As to the effects of adrenin on the bone circulation in the intact animal

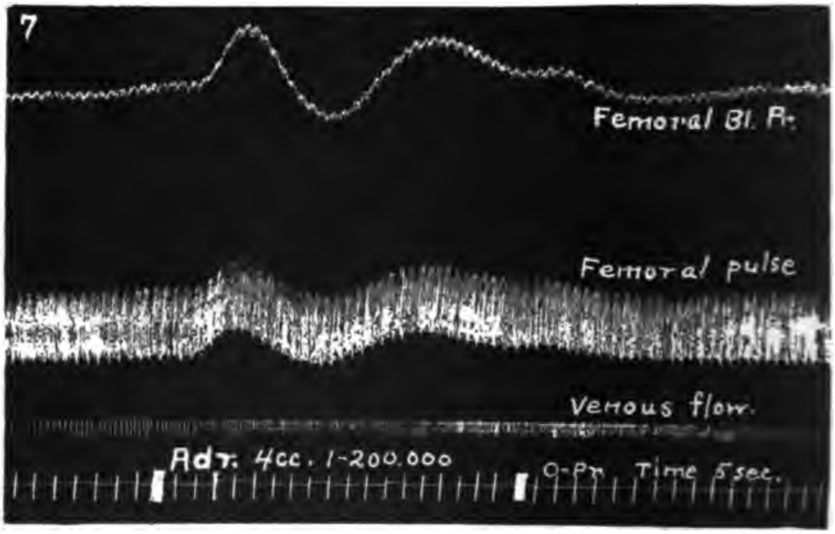

Fig. 7. Outflow from a vein of a muscle augmented by adrenin, pressor infusion. Registered by drops. No reduction.

apparently nothing is known. Recent work by Drinker and Drinker (23) has shown that when it is perfused through the isolated bone the rate is decreased.

Capps and Mathews (24) have shown that adrenin injections of the magnitude used in our experiments have little or no effect on venous blood pressure. Measuring the rate of outflow from an open vein, therefore, under various conditions of adrenin administration should afford a reliable index of the effects of such administration on the blood flow in an intact animal.

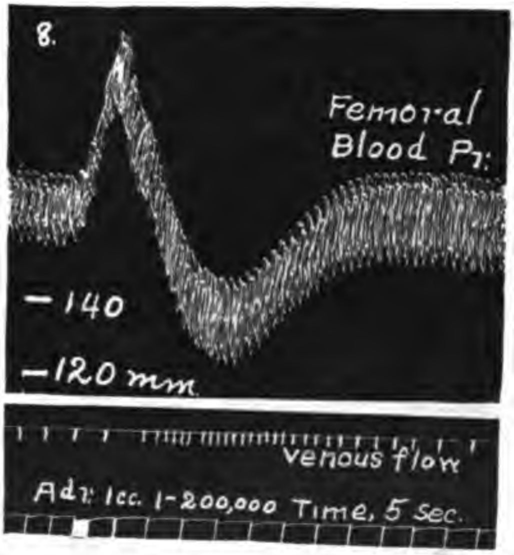

Fig. 8. Outflow from a vein of a muscle augmented during the depressor phase of reaction to adrenin. Registered by drops. No reduction.

The researches were accordingly extended in the following way: A femoral vein was exposed in the groin. One of its branches coming from an underlying muscle was further isolated and in it an oiled cannula was tied. The outflow was recorded by drops on a kymograph drum by means of a key and signal magnet. At the same time arterial pressure was recorded as in the earlier experiments of the series. Adrenin was administered through the femoral vein of the opposite leg both by instantaneous injections and by infusions at various rates. Then a cutaneous branch of the same femoral vein was cannulated and the experiments repeated. The conditions therefore, were in every way comparable except that the outflow in one case was from the muscle and in the other from the skin.

Effect in muscular branches. Figures 7 and 8 show the results secured in case of the muscular branches. The rate of outflow was increased, sometimes slightly and sometimes more than 100 per cent. That the effect was active rather than passive is shown by both figures. In figure 7 the outflow continued at an increased rate for half a minute after the blood pressure had returned to normal. In the case shown in figure 8 an augmented outflow and a lowered arterial pressure occurred simultaneously. Indeed the reduced arterial pressure in such cases is probably due very largely to exactly this augmented outflow from the vessels of the muscles. Similar results were obtained throughout the series both with large and small doses. It is evident, therefore, that adrenin exercises a selective vasodilator effect in skeletal muscle.

Effect in cutaneous branches. The effect in the skin is exactly the opposite of that in muscles. Figure 9 shows a typical result when the cannula is inserted in a cutaneous branch. After a momentary acceleration of the flow, due supposedly to the propulsive effect of contraction of the peripheral vessels the outflow is markedly decreased. Infusion experiments showed that the retardation persisted as long as the administration of adrenin was continued and for about half a minute thereafter. Here again the fact that the venous outflow was retarded irrespective of the changes of arterial pressure shows that the effects were not passive. In no case was adrenin observed to cause an augmented outflow from the cutaneous vessels. A source of error that not infrequently gave results that might be interpreted as due to vasoconstriction both in the muscular and cutaneous branches was clotting of the blood in the cannulas. In all cases in which the rate of flow failed to return approximately to normal when the adrenin was discontinued the records were discarded.

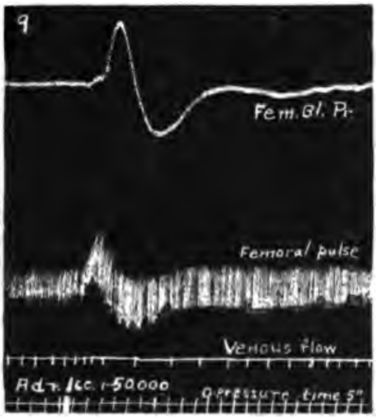

Fig. 9. Outflow from a cutaneous vein decreased by adrenin injection. Registered by drops. (Reduced to $\frac{2}{3}$.)

DISCUSSION

From the foregoing observations it is obvious that in an intact animal adrenin in any general sense is neither a vasoconstricting nor a vasodilating agent. It is one or the other depending upon what vessels are involved. In some cases, as in the lungs, the effect depends also upon the dosage (4). Neither can one generalize to the effect that the splanchnic vessels are constricted while the peripheral vessels are dilated. In the peripheral circulation are included not only the skin but also other less extensive structures that respond by constriction while observations not yet reported have shown that the gut is often dilated. The effect of adrenin in such a structure as a limb is a composite one, the limb as a whole either contracting or expanding depending upon whether vasodilatation in the muscle or vasoconstriction in the skin predominates. The fact that the muscular tissues form the greatest mass in the trunk and limbs would seem to account for the fact that adrenin passed through the extra-splanchnic circulation causes a fall in arterial pressure.

It is probable that the selective vasodilator effect of adrenin in skeletal muscle accounts to a considerable degree for its favorable influence in improving contractile activity as well as hastening recovery from fatigue.

This selective vasodilator effect may account for certain other phenomena otherwise difficult of explanation that have been described. The waves of augmented activity that appear in ergographic curves when the experiments are carried to unpleasant lengths may possibly be due to adrenal discharges. The same explanation is more plausible in case of a phenemenon observed by Hill and Nabarro (25). They measured the outflow from the femoral vein of a dog during an epileptic fit and found it several times greater than during rest. Periods of excitement are known to be accompanied by augmented adrenal discharge and this is probably true of the type of excitement studied by Hill and Nabarro.

SUMMARY AND CONCLUSIONS

1. Adrenin injected by vein in the intact animal generally causes in all dosages a constriction of the limbs. Occasionally, however, expansion occurs.
2. Removing the skin converts the contraction to expansion.
3. Adrenin in both pressor and depressor dosages causes an increase in the venous outflow from muscles.
4. Adrenin, therefore, causes active vasodilatation in the muscles.
5. Adrenin causes decreased outflow from the skin hence vasoconstriction in the cutaneous vessels.
6. The effects of infusions and of instantaneous injections are qualitatively similar.
7. The local vasodilator effects of adrenin probably account for at least a part of its beneficial effect in active and fatigued muscle.

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THE EFFECT OF PITUITARY EXTRACT ON THE FROG'S ESOPHAGUS

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The physiology and to a limited extent the pharmacology of the frog's esophagus have been investigated by various experimentalists chief among whom may be mentioned Botazzi and Gruenbaum (1) and Stiles (2). The former studied sections of the organ suspended in a moist chamber and found the longitudinal strata to be rhythmically active and to respond to drugs essentially like the mammalian intestine. Stiles on the other hand studied the transverse musculature of the opposite extremities of the esophagus suspended in Ringer's fluid and observed that, in comparison with one another, the pharyngeal end had a more active rhythm but a less extensive amplitude than the gastric.

Relative to the effect of pituitary extract on the gastro-intestinal system of mammalia, an extensive literature has accumulated during the past decade (4). Among other phenomena, our attention has been several times called to the presence of a depressor substance whose effect is to markedly decrease the tone and rhythmic activity of the intestines of the rabbit and the dog. The chief students of this depressor action are:

1. Bayer and Peter (3) who showed that certain preparations of pituitary body (they used principally the "Pituitrin" of Parke, Davis and Company) decreased the tone and the contractions of the rabbit's intestine.

2. Shamoff (4) who showed that this depression of the excised rabbit's intestine was produced by most of the extracts available to him, but not by Armour's pituitary liquid. He did not use "Pituitrin."

(3) Hoskins (5) who has called attention to this depressor action on the dog's intestine in situ and thinks that the present commercial preparations vary in the content of depressor substance, the variation probably resulting from differences in the mode of production.

As this experimental work was done during the early months of summer, it may be of importance to make a brief statement in regard to the care and condition of the frogs from which the tissue was taken. The frogs were kept out of doors in a large tank provided with an earth covered platform placed just above the surface of the water and shielded from the direct rays of the sun by a canvass covering. They were fed on earth worms; fresh water was admitted once or twice per day; about once per week a little salt and lime were added to the water. The whole batch remained in excellent condition throughout the entire period covered by the experiments.

I used, in these experiments, sections of the freshly excised esophagus taken from pithed frogs and suspended in oxygenated Ringer's Fluid. These sections were as follows: (1) Longitudinal from the whole length of the organ; (2) rings from near the pharynx and from near the stomach; and (3) longitudinal segments from near the pharynx and from near the stomach.

The method of suspending and registering was similar to that used by Stiles. One end of the section was fastened below to a glass rod bent so as to dip into a small conical "tissue-bath" which itself was surrounded by a water jacket equipped with a constant temperature regulator. The free end of the section was attached by means of a silk thread to a light lever which was adjusted to write on a smoked surface. All preparations of tissue were heavily weighted to prevent the appearance of troublesome tone changes.

The preparations of pituitary extract employed were Armour's pituitary liquid and Parke, Davis and Company's pituitrin. These were used in strengths of from 1:125 to 1:300. This would be in terms of the fresh gland substance from 1:9000 to 1:22,000 (6), approximately, which is very much more dilute than that used by Bayer and Peter on the rabbit's intestine.

1. *Longitudinal sections of the whole esophagus.* Application of pituitary extract was followed by a decrease in tone, rhythm, and amplitude of contraction. In most cases, the contractions ceased entirely for from one to three minutes and then resumed with a more rapid rhythm but less extensive amplitude. The tone did not tend to be restored during the continuance of the drug in the tissue-bath, though the amplitude of the contractions sometimes approximated the normal.

Application of adrenaline, which affects the frog's esophagus as it does the rabbit's intestine, deepened the depression produced by pituitary extract. Pilocarpine and barium chloride both counteracted the depression.

The tissue did not seem to suffer any permanent injury from contact with the drug, for the tone and contractions became normal almost immediately after fresh fluid had been put into the tissue-bath. Repetition, now, of the application of the extract was followed by the same series of events as followed the initial use of the drug.

2. *Portions of the opposite ends of the esophagus.* Having observed the above recorded effects on longitudinal sections of the whole esophagus, it seemed important in view of the difference in rhythmicity at the opposite extremities of the organ (Stiles) to determine whether pituitary extract would show differences as regards its effect at the two ends of the organ. Accordingly, sections from the opposite extremities were compared with one another, (a) circular gastric and pharyngeal and (b) longitudinal gastric and pharyngeal. In making the comparisons, the sections were suspended in separate baths but preferably in the same bath where conditions would be identical.

(a) The circular muscle of both the gastric and pharyngeal ends was depressed. The depression of the pharyngeal end was tardily produced as compared with that of the gastric, the latter responding almost instantaneously with decrease in tone, rhythm, and extent of contraction, the former chiefly with decrease in tone.

(b) The longitudinal muscle of both the gastric and pharyngeal ends was depressed. The depression at the gastric end was very marked, while that at the pharyngeal was only slight.

3. *Influence of temperature.* Portions of the organ were also tried at different temperatures between 22°C. and 38°C. While as has been shown by previous investigators the tissue is more active at certain temperatures than at others, the reaction to pituitary extract was not altered, the tissue responding to the drug proportionately as actively at low as at high.

4. *Influence of oxygen.* It has been stated by certain authors that the depressor action may be augmented in the presence of oxygen due to the ease with which the pressor substance oxidizes. In view of this, experiments were performed in which the supply of oxygen was cut off completely. Of course, under these circumstances, the tissue tended to lose tone and to be less active than when oxygen was supplied, but on the whole the reaction to pituitary extract was unchanged and restoring the supply of oxygen produced no effect other than could be attributed to the oxygenation (see fig. 1).

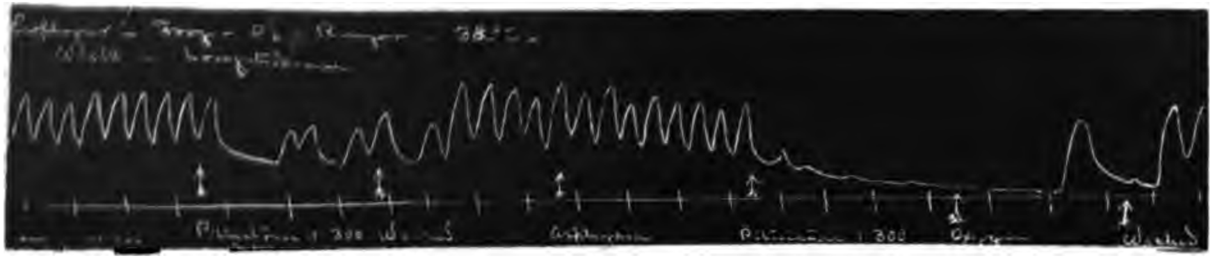

Fig. 1. Whole esophagus, showing the effect of pituitary extract on the longitudinal muscle deprived of oxygen. Time, one minute.

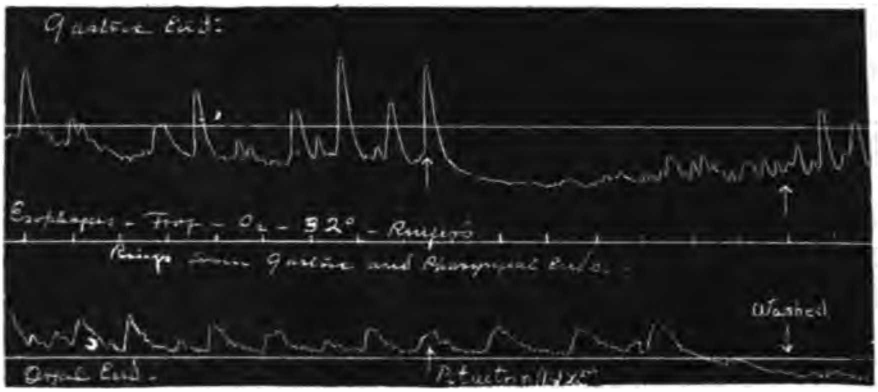

Fig. 2. Simultaneous records of the circular muscle of the opposite ends of the esophagus, showing the tardiness of reaction of the pharyngeal end as compared with that of the gastric end. Time, one minute.

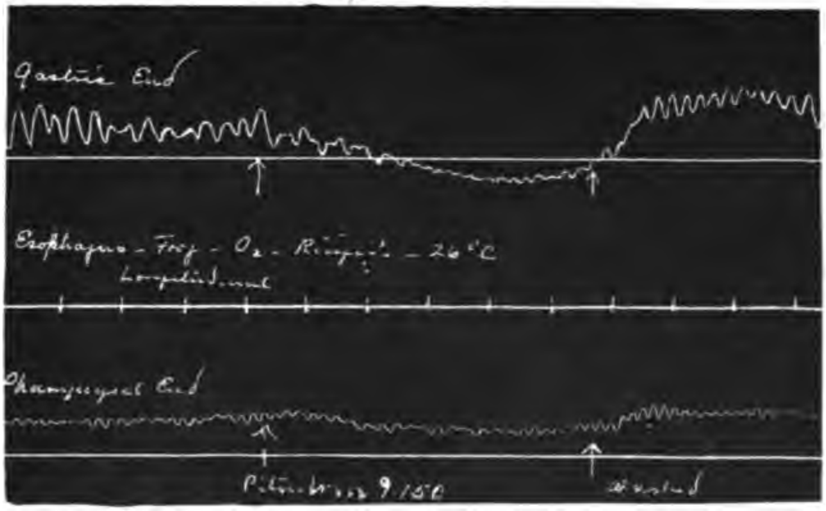

Fig. 3. Simultaneous records of the longitudinal muscle of the opposite ends of the esophagus, showing the slight effect of pituitary extract on the pharyngeal end. Time, one minute.

5. Further, endeavor was made to determine whether the depressor substance was used up in the process of depressing the tissue. In making this determination, two preparations (a and b) were suspended in separate baths. To one of these (a) pituitary extract (1:300) was applied and after the resulting depression had lasted for five minutes, the fluid was drawn off and applied to the other section (b). Depression followed in (b) as promptly as in (a) and the quantitative effect was the same.

SUMMARY

1. Pituitary extract depresses both the circular and the longitudinal musculature of the frog's esophagus.

2. In this depression, the tone, the rhythm, and the amplitude of the contractions are all affected simultaneously.

3. The depression is shown more markedly at the gastric end than at the pharyngeal.

4. Variations in the supply of oxygen, or even its total suppression, and variations in temperature do not modify the depression.

5. The depressor substance does not seem to be destroyed in the process of depressing the tissue.

6. Armour's pituitary liquid and Parke, Davis and Company's pituitrin react essentially alike on the frog's esophagus.

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STUDIES ON THE GROWTH OF MAN

III. The Growth of British Infants During the First Year Succeeding Birth

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1. OBJECT OF THE INVESTIGATION AND SOURCES OF DATA

While we now possess a fairly accurate and extensive knowledge of the normal curve of growth of certain animals, particularly the white rat and the white mouse, it is a regretable fact that we are not yet in possession of equally reliable and extensive data concerning the growth of human beings. While we know that growth in human beings as in mice takes place in three "cycles" or periods of alternately rapid and slow growth (1), we do not yet possess, for any one race of mankind, a detailed knowledge of the extent and characteristics of each of these cycles. We possess in several instances very extensive knowledge of portions of the total curve, and if it were permissible to add together data obtained from different countries we would possess a sufficiency of information to enable us to construct in detail the entire growth-curve of man. But the most superficial comparison of data from different countries, as for example those of the British Anthropometric Committee (2) with those obtained by Quetelet in Belgium (3) at once reveals the fact that the growth-curves of different races of man or even of similar races inhabiting different localities (4) are by no means identical and that parts of the growth-curve constructed from measurements made in France for example cannot therefore be legitimately regarded as continuations of other parts of the human growth-curve constructed from measurements made in England.

As regards the inhabitants of [illegible] reports of the Anthropometric [illegible] on Physical Training (Scotland) [illegible] ment (7), the medical officer of health [illegible] chief medical officer of the [illegible] picture of their growth from the beginning [illegible] Similar data regarding growth [illegible] ever, to be lacking.

The medical advisors of the various [illegible] Britain appear usually to regard the normal [illegible] as representing the growth of British infants [illegible] succeeding birth, and charts representing this standard [illegible] displayed in the offices of these organizations [illegible] poses of comparison upon the growth-charts of the [illegible] care. I was unable, however, by inquiry [illegible] medical advisors of these institutions in London [illegible] of this standard, and therefore addressed [illegible] George Newman, who very kindly referred [illegible] on Infant Mortality (20). In that work [illegible] of the "Standard" are supplied, the source of the [illegible] which the charts are constructed is nowhere [illegible] indeed it be upon page [illegible] where the author [illegible] ard has been used in the Finsbury [illegible] and the charts appearing in the present work [illegible] scale."

The standard of infant growth employed [illegible] fore appear to have originated in France. [illegible] marked influence of locality upon human growth [illegible] discrepancy between the weights of British [illegible] and those of infants born in the British [illegible] the influence, to which reference has been [illegible] growth, it is very evident that a French [illegible] relied upon as representing the normal growth [illegible] marked discrepancies between the average [illegible] and those indicated by the "Newman [illegible] anticipated. Moreover the Newman [illegible] between the sexes, whereas as the above [illegible] servations upon animals show, the growth [illegible] even during the first growth-cycle differs [illegible] siderably.

That marked discrepancies exist between the actual normal weights of British infants and the Newman Standard weights has been pointed out by Pooler (12).

It appeared, therefore, in order to construct this portion of the growth-curve of the inhabitants of Great Britain, necessary to obtain fresh estimates of the average weights of children at monthly intervals during the first two years succeeding birth. I accordingly, in the spring of 1914, addressed myself to the medical advisors and other officials of the various child-welfare institutions in London, Leeds and Bristol and in several instances obtained permission, which was very greatly appreciated, to obtain access to and to copy the valuable data collected by these institutions during the execution of their activities. For assistance in obtaining these data I am especially indebted to the following persons to whom I wish to take this opportunity of expressing my obligations:

Drs. Eric Pritchard, David Forsyth and R. C. Jewsbury in London, Drs. John Robertson and H. W. Pooler in Birmingham, Dr. Leslie Mackezie in Edinburgh, Dr. James Smart in Aberdeen, Mrs. Ogilvie, honorary secretary of the Chelsea Health Society; Miss Margaret Horn, honorary organizer of the city of Westminster Health Society. Mrs. Kitson Clark, president of the Leeds Babies Welcome, Miss E. Mozley, honorary secretary, Leeds Babies Welcome; Miss M. Curtis, superintendent Leeds Babies Welcome and F. M. Townsend, chairman of the Bristol Infant Welfare Association.

The data reported in this article were obtained from the records of the Pimlico Road and Golden Square branches of the Westminster Health Society and the Chelsea Health Society in London and from the Leeds Babies Welcome. The data obtained from other cities proved to be too fragmentary to utilize in statistical studies of this type.

In the case of the London institutions mentioned I was given the opportunity of personally inspecting the methods employed in weighing and examining the children and in keeping the records. In certain institutions which I visited no records of sex were kept and their data could not, therefore, be utilized. Other institutions only weighed the infants very approximately, and these data likewise were rejected. In the institutions mentioned, however, the sex of the infant is indicated upon the record and the weights are recorded to the nearest ounce avoirdupois. The infants were voluntarily brought by their mothers to the offices where a medical advisor and nurses or other trained officials were in attendance, the weight and condition of the child being noted at each visit upon its record. The weights thus recorded were tabulated and averaged in the manner employed in the investigations previously reported (13). The weights of all infants reported as suffering from measles, whooping cough, chicken-pox, scarlet fever, diphtheria, pneumonia, bronchitis (unless specifically noted as "slight"), rachitis (unless specifically noted as "slight") and marasmus were excluded upon and succeeding the date upon which the condition was first noted. Data concerning twins and malformed children were likewise excluded and only children stated to be of British parentage on both sides and wholly or partially breast-fed (14) were accepted as sources of data. I was unable to personally visit the Leeds Babies Welcome, but the growth charts of a large number of infants were sent to me through the courtesy of the officials. The data as to disease included in these charts were not so full as in the London Records to which I had access and therefore although the charts furnished to me were specified as records of "normal children" it is possible that the selection was somewhat less rigorous in the case of the Leeds infants than in the case of the London infants. This may in part account for the slight discrepancy between the averages from the two sources during the first year of post-natal growth and for the very marked divergence of the two groups of children during the second year, to which attention will subsequently be drawn.

The data reported are exclusively ante-bellum, and were for the most part derived from records taken in the years 1911–1914. The delay in publication of the results arose from pressure of other work and from the large number of arithmetical computations involved.

2. RESULTS

The following tables 1 and 2 were the results obtained for infants during the first year of post-natal growth. The averages headed "England" are obtained by combining the data from London and Leeds, the variabilities at each age being calculated in the manner usually employed in statistical investigations (15).

The values of the Newman Standard are derived from the chart [illegible] page [illegible] in Newman's Infant Mortality. On comparing the standard with the results of these weighings it will be obvious that, as already noted by Pooler, the Newman Standard is considerably [illegible] infants.

TABLE 1

Males

AGE IN MONTHS	LONDON		LEEDS		ENGLAND			NEWMAN STANDARD
	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Variability	
							per cent	
1	48	149.0	38	144.4	86	147.0	17.0	123
2	69	172.9	48	163.4	117	169.0	16.4	142
3	69	197.1	65	190.3	134	193.8	15.8	161
4	48	227.3	66	212.3	114	218.6	15.9	180
5	52	244.8	72	225.8	124	233.7	14.7	204
6	38	266.0	67	243.7	105	251.8	15.1	228
7	28	283.4	62	262.0	90	268.6	14.9	244
8	34	289.4	60	268.7	94	276.2	13.1	255
9	24	286.7	61	281.2	85	282.8	13.8	268
10	21	320.2	46	290.4	67	299.7	13.4	282
11	24	313.8	37	296.6	61	303.4	15.3	295
12	19	342.6	41	301.2	60	314.3	12.6	308

TABLE 2

Females

AGE IN MONTHS	LONDON		LEEDS		ENGLAND			NEWMAN STANDARD
	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Number weighed	Average weight in ounces	Variability	
							per cent	
1	44	143.7	36	142.4	80	143.1	14.4	123
2	67	159.1	47	160.0	114	159.5	15.0	142
3	66	179.1	57	181.0	123	180.0	15.6	161
4	56	201.9	48	202.2	104	202.1	15.0	180
5	41	221.2	49	214.5	90	217.6	14.5	204
6	34	240.3	49	232.0	83	235.4	13.4	228
7	30	258.7	46	248.6	76	252.6	14.6	244
8	33	268.5	46	251.2	79	258.4	14.0	255
9	27	268.1	53	264.0	80	265.4	14.0	268
10	22	274.2	35	272.7	57	273.3	11.6	282
11	22	301.5	28	277.4	50	288.0	12.5	295
12	25	304.7	31	273.6	56	287.5	11.9	308

ability is therefore greatest when the velocity of growth is greatest, a fact in harmony with data obtained in studying the growth of the white mouse (18).

It appears probable that high variability of weight, dimensions or other characteristics constitutes on the whole a condition unfavorable to the average welfare of any given group of organisms, since a high degree of variability implies more frequent possibility of overstepping physiological limits. It appears not unlikely therefore that the higher variability of male infants at birth (19) and subsequently to birth is correlated with the higher mortality among male infants at these ages (20).

3. THE FORMULATION OF THE CURVE OF GROWTH FOR BRITISH INFANTS DURING THE FIRST TWELVE MONTHS SUCCEEDING BIRTH

I have shown in previous communications (21) that the relationship between weight and age for any single growth-cycle is the same as that which subsists between the extent of transformation and the time in an autocatalysed chemical reaction, that is to say, a reaction one of the products of which accelerates it. The formula expressing this relationship is:

$$\log \frac{x}{A-x} = K\,(t-t_1)$$

where A is a constant (= the maximum weight attained by the particular growth-cycle under consideration), t_1 is a constant (= time at which the growth-cycle is half-completed) K is a constant (= kA where k is the specific velocity-constant and A has the value assigned above) and x and t are the weight and time respectively.

Applying this formula to the above data, utilizing for this purpose the methods and tables described in my "Tables for the Computation of Curves of Autocatalysis, with Especial Reference to Curves of Growth" (22) we find that for British males, during the first nine months of extra-uterine growth[2] the equation becomes:

$$\log_{10} \frac{x}{318-x} = 0.127\,(t-1.46)$$

inserting the observed values of t and computing therefrom the corresponding values of 0.127 $(t-1.46)$ we can find, by referring to the above cited tables, the corresponding values of $\frac{x}{318}$ and, consequently, compute the "calculated" or theoretical values of x. The observed and calculated values are compared in table 3 and illustrated in figure 1, the smooth curve representing the calculated curve and the dots the weights actually observed. In this comparison the weights at ten, eleven and twelve months are included although the theoretical curve is not computed to fit these values. In the fifth and sixth columns of the table the divergencies between the actual and the theoretical weights are compared with the "probable errors" of the observed average weights computed in the usual manner from the variabilities and the numbers of infants weighed at each age.

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[2] In computing the constants of the curve the data for the tenth, eleventh and twelfth months are not employed, since there is a possibility that in this region the curve of the first cycle may be distorted by growth due to the beginnings of the second cycle.

TABLE 3

Males

AGE IN MONTHS	WEIGHT IN OUNCES		DIVERGENCE FROM OBSERVED AVERAGE	PROBABLE ERROR OF OBSERVED AVERAGE
	Observed	Calculated		
1	147.0	148.2	+1.2	±1.8
2	169.0	171.4	+2.4	±1.7
3	193.8	194.3	+0.5	±1.8
4	218.6	215.6	−3.0	±2.2
5	233.7	234.7	+1.0	±2.1
6	251.8	251.5	−0.3	±2.5
7	268.6	265.5	−3.1	±2.8
8	276.2	277.0	+0.8	±2.5
9	282.8	286.5	+3.7	±2.8
10	299.7	292.8	−6.9	±3.3
11	303.4	299.6	−3.8	±4.0
12	314.3	304.0	−10.3	±3.4

The corresponding equation for the first growth-cycle in females is:

$$\log_{10}\frac{x}{312-x} = 0.106\,(t-1.54)$$

the comparison of the observed and calculated values of x follows in table 4 and is illustrated in figure 2.

It will be seen that the agreement between the observed and calculated values of x is excellent, the only important divergence being in the males at twelve months when, as explained above, deviations due to the intrusion of the second growth-cycle are to be anticipated.

TABLE 4

Females

AGE IN MONTHS	WEIGHT IN OUNCES		DIVERGENCE FROM OBSERVED AVERAGE	PROBABLE ERROR OF OBSERVED AVERAGE
	Observed	Calculated		
1	143.1	145.7	+2.6	±1.5
2	159.5	164.7	+5.2	±1.5
3	180.0	183.5	+3.5	±1.7
4	202.1	201.6	−0.5	±2.0
5	217.6	218.1	−0.5	±2.2
6	235.4	233.4	−2.0	±2.3
7	252.6	246.8	−5.8	±2.8
8	258.4	258.6	+0.2	±2.7
9	265.4	268.6	+3.2	±2.8
10	273.3	276.7	+3.4	±2.8
11	288.0	283.9	−4.1	±3.4
12	287.5	289.5	+2.0	±3.1

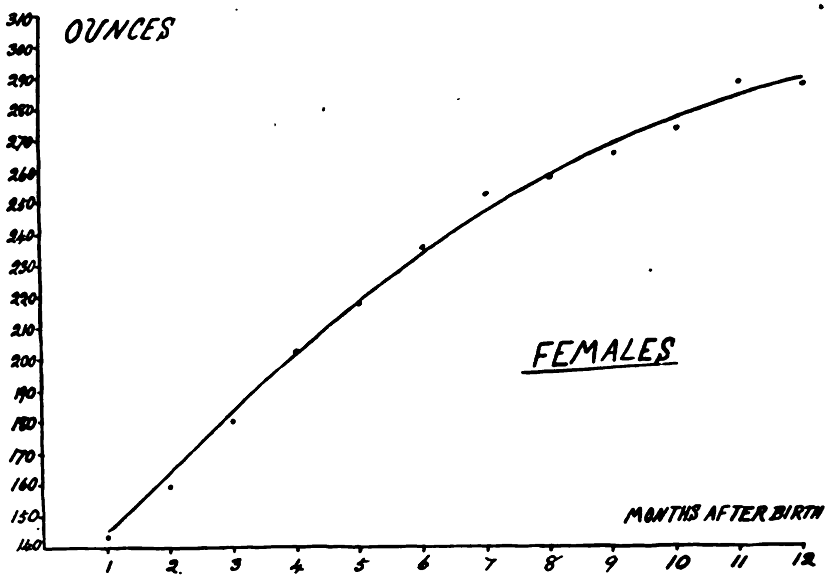

FIGURE 2.

In the following table (5) the values of the parameters $A, k\left(=\frac{K}{A}\right)$ and t_1 for British infants and for Australian infants of British descent (23) are compared:

TABLE 5

	MALES			FEMALES		
	A	$k \times 10^6$	t_1	A	$k \times 10^6$	t_1
British	318	399	1.46	312	340	1.54
Australian	341.5	398	1.66	350	317	2.47

It will be observed that while so fundamental a difference as that of sex has little effect upon the absolute magnitude ($= A$) of the first growth-cycle, environment has a pronounced effect, since one or two generations of the Australian (presumably more favorable) environment has resulted in an increase of from 7 to 12 per cent in the amplitude of the cycle. The specific velocity of the growth-process, however, ($= k$) is comparatively unaffected by environment, the identity of the two values for males being particularly striking, while it is decidedly less for females than for males. Corresponding with these correlations we find that the period of half-completion of the cycle (and maximum velocity of growth) is later in females than in males and in Australian than in British infants.

The form of the curve of growth in infants is therefore determined by two separate factors; the one, analogous to the absolute mass of reacting substances in a chemical reaction, is dependent upon environment and probably upon the abundance or deficiency of the habitual dietary; while the other, analogous to the specific velocity of a chemical reaction, is relatively if not absolutely independent of environmental or nutritional conditions and, being expressive of the nature of the growth-process itself as distinguished from the availability of materials for growth, is distinctively modified by sex and probably also by race.

4. SUMMARY

1. From data supplied by infant welfare associations in London and Leeds the curve of growth of British infants during the first twelve months succeeding birth has been constructed.

2. The Newman Standard, hitherto largely employed as a standard curve of growth of British infants, is defective in two particulars. In

the first place it makes no distinction between the sexes, although the growth-curves of the two sexes differ in very material particulars, and in the second place, especially during the early months, the weights indicated by the Newman Standard are considerably sub-normal.

3. The variability of male infants is somewhat greater than that of female infants. The variability of both sexes is greatest during the early months, or period of most rapid growth, and decreases towards the later part of the first year, when the rate of growth is also decreasing.

4. The curve of autocatalysis correctly represents the growth of British infants during the first nine or ten months succeeding birth.

5. Comparing the characteristics of the growth-curves of British infants with those of the growth-curves of South Australian infants of British descent, we find:

i. The absolute magnitude or amplitude of the first growth-cycle in man is unaffected by sex, but is decidedly affected by environment, being increased in the Australian environment by from 7 to 12 per cent.

ii. The specific velocity of the growth-process is, on the contrary, very little affected by environment and markedly influenced by sex, being, in both environments, decidedly lower in the females than in the males.

iii. The period of half-completion of the cycle is later in females than in males and in Australia than in England.

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STUDIES ON THE GROWTH OF MAN

IV. THE VARIABILITY OF THE WEIGHT AND STATURE OF SCHOOL CHILDREN AND ITS RELATIONSHIP TO THEIR PHYSICAL WELFARE

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In any fortuitously selected group of animals or human beings, the individuals comprising the group will be found to vary in greater or lesser degree from the type or average which the group collectively represents. When a large proportion of the individuals comprising the group differ from the average to a considerable degree we say that the group or type is highly variable, while when the converse holds good, that is when but few of the individuals comprising the group depart widely from the average, then we consider the group or type to be but slightly variable. It is evident that if a measure of variability can be conveniently obtained it represents a factor or group of factors which may conceivably be affected by environmental or physiological conditions in a distinctive manner. In comparative studies of growth under varying environmental or dietetic conditions a knowledge of the variability of the organisms studied is essential in order to be able to determine the deviations from the average which may be regarded as normal, and those which on the contrary must be regarded as abnormal and attributable to the experimental conditions under which we have placed the individuals which display them.

The measure of variability which is commonly employed by statisticians is that deviation from the average which one-third of the individuals display (1). This may conveniently be expressed as a *percentage* deviation from the average weight or stature or other measurement under consideration.

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[1] I am indebted to Mr. R. M. Jewett for assistance in the computation and compilation of the data.

Investigations upon the growth of the white mouse (2) have shown that the variability in weight increases when the velocity of growth increases, decreases when the velocity of growth decreases, attains a maximum when the rate of growth attains a maximum, and attains a minimum, when the rate of growth sinks to a minimum, i.e., when the animal is fully grown. It has also been shown (3) that similar phenomena accompany the first year of post-natal growth in infants the variability of weight being relatively high during the early months when growth is relatively rapid, and relatively low during the later months when growth is relatively slow. Variability in weight is therefore affected in a distinctive manner by internal factors, namely the processes underlying and controlling the rate of growth. That variability may likewise be affected by dietetic factors is shown by the remarkable increase in the variability of mice when fed with unusual amounts of cholesterol (4); while, as I am about to show, the various factors comprised in the external environment may also exert an effect upon the variability of weight and stature in man.

As stated in the preceding article, it would appear probable that a high degree of variability is a condition which is disadvantageous to the collective welfare of the individuals which display it, since a high frequency of marked departures from the mean implies a more frequent overstepping of physiological limitations. Conversely, other conditions being equal, a disadvantageous environment might be expected to cause an increase in variability through enhancing preëxisting differences or at any rate preëxisting subnormality. It is, however, to be borne in mind that disadvantageous environmental conditions may cause a retardation of the growth of young animals which in its turn might be expected to lead to a *decrease* in variability. Where the disadvantageous conditions operate at a period of rapid growth, or of growth of which the rate is fluctuating, the retardation of growth due to unfavorable environmental conditions might be expected to lead to a diminution or subnormality of variability, while the same conditions, pressing upon an adult animal, or an animal growing at a relatively slow or steady rate, might be expected to lead to an enhancement or supernormality of variability.

From yet another point of view the investigation of variability is of importance. The growth or the physical well-being of an individual may be estimated in a variety of ways and by means of a variety of measurements of which weight and stature are those most frequently employed. If we desire to obtain a reliable criterion of abnormality we shall do well to select for our purpose the measurement which is normally found to be least variable, since relatively small deviations from the normal are that case fraught with significance which does not attach to similar deviations from the normal displayed by a more variable characteristic. On the other hand if we desire to study in detail the effects of fluctuations in the diet, environment or physiological condition upon the dimensions of an individual, then we shall do well to investigate the more variable characteristics, since, from their high normal variability we may infer that they are exceptionally affected by environmental or other fluctuations. A previous knowledge of the relative normal variabilities of different physical dimensions in man or animals is therefore of service in enabling us to determine beforehand which type of measurement will be most likely to display the effects or correlations for which we are seeking.

The present investigation was undertaken with a view to ascertaining (*a*) the relative variability of weight and stature in school children and (*b*) the influence of environment upon the variabilities of these dimensions.

Through the courtesy of the director of health of the Oakland School Department, Dr. N. K. Foster, to whom I desire to express my very deep indebtedness, I have been permitted access to his very valuable and extensive records of the physical dimensions and welfare of the school children in the various schools in the city and environs of Oakland, California.

The records of fifty children of each sex and age, from 6 to 14 years inclusive (i.e., seventh to fifteenth year) were selected at random, excluding only seriously deformed individuals, from among the records of three "average" schools, that is, schools situated in localities inhabited by persons of average means and welfare. The heights and weights of these children were averaged and the variabilities of these dimensions determined in the usual manner (1).

The results are indicated in tables 1 and 2 and displayed graphically in figures 1 and 2.

From these results it is evident that the rate of growth in weight in both sexes increases continuously from the seventh to the fifteenth year and that the variability in weight undergoes a parallel increase during this period. There is a manifest tendency for maxima of variability in weight to coincide with maxima of yearly increment. The stature, on the other hand, increases during this period at an almost uniform rate and the variability of stature is correspondingly uniform.

TABLE 1

Males

AGE IN YEARS LAST BIRTHDAY	AVERAGE WEIGHT IN POUNDS	YEARLY INCREMENT	VARIABILITY	AVERAGE STATURE IN INCHES	YEARLY INCREMENT	VARIABILITY
			per cent			*per cent*
6	46.0		12.2	46.1		5.6
7	50.7	4.7	12.1	47.9	1.8	5.5
8	55.4	4.7	13.5	50.3	2.4	4.8
9	64.0	8.6	13.7	52.2	1.9	4.9
10	67.1	3.1	12.0	53.5	1.3	5.1
11	75.9	8.8	12.6	56.0	2.5	3.6
12	81.3	5.4	15.7	56.7	0.7	5.1
13	92.6	11.3	17.3	59.6	2.9	5.2
14	103.6	11.0	20.3	61.7	2.1	5.7

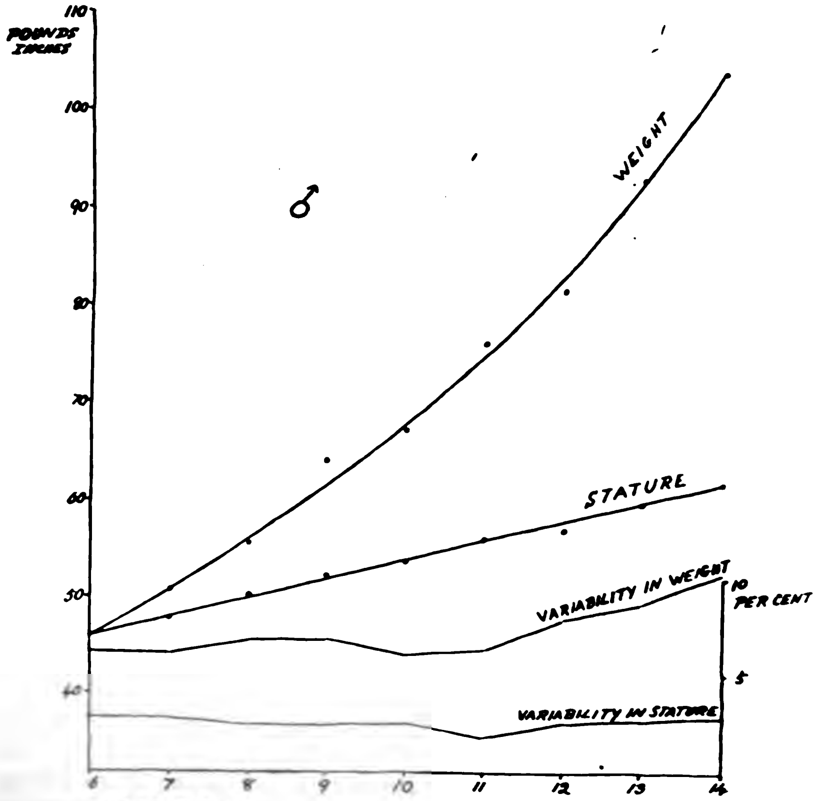

FIGURE 1

TABLE 2

Females

AGE IN YEARS LAST BIRTHDAY	AVERAGE WEIGHT IN POUNDS	YEARLY INCREMENT	VARIABILITY	AVERAGE STATURE IN INCHES	YEARLY INCREMENT	VARIABILITY
			per cent			*per cent*
6	44.4		11.9	45.3		5.8
7	49.4	5.0	12.0	47.7	2.4	4.4
8	53.5	4.1	13.4	49.8	2.1	4.1
9	59.7	6.2	12.8	51.4	1.6	4.6
10	66.1	6.4	14.2	54.2	2.8	4.8
11	75.2	9.1	20.3	55.5	1.3	5.8
12	80.7	5.5	17.1	58.0	2.5	5.2
13	97.3	16.6	20.5	61.1	3.1	4.7
14	105.6	8.3	13.6	62.4	1.3	5.4

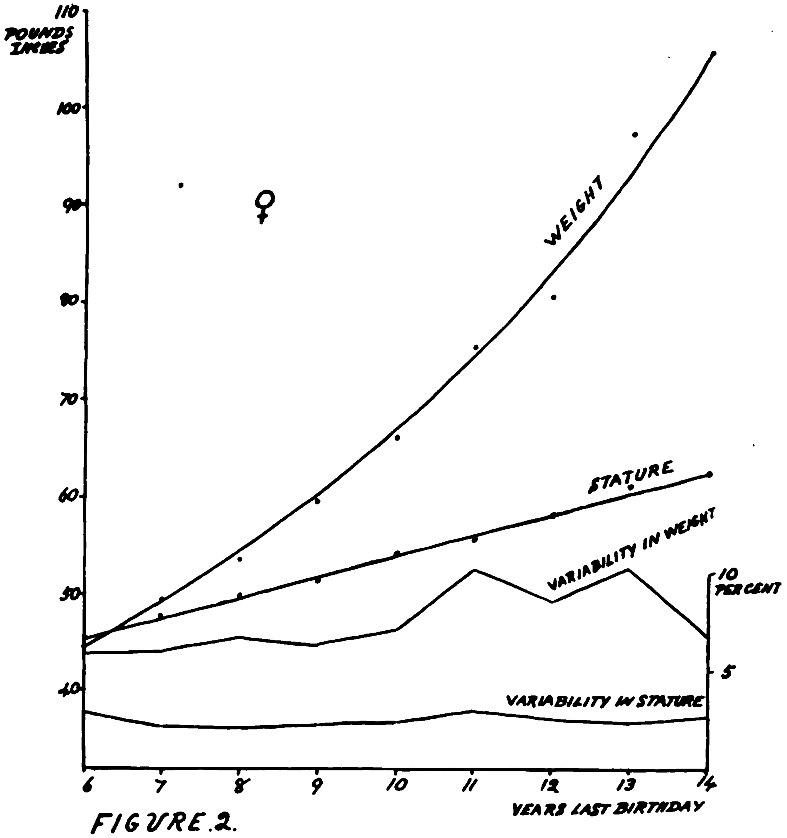

FIGURE.2.

The variability in stature is much less than the variability in weight, from which we may infer that as a criterion of abnormality the measure of stature is more reliable than that of weight, while as a sensitive indicator of the effects of environmental or dietetic fluctuations the measure of weight is to be preferred to that of stature.[2]

In order to directly estimate the influence of environment upon variability of weight and stature we also selected at random the records of fifty children of each sex and of 8 years of age from among the records of children attending a school which was situated in a relatively poor industrial district, inhabited mainly by factory operatives, etc., while another group of records of fifty children of each sex and of 8 years of age was similarly selected from among the records of children attending a school situated in a relatively wealthy residential section of the city. The results are summarised in tables 3 and 4, while by

TABLE 3

Males

SCHOOL	AVERAGE WEIGHT IN POUNDS	VARIABILITY	AVERAGE STATURE IN INCHES	VARIABILITY	PERCENTAGE OF CHILDREN HAVING	
					Infected adenoids	Adenoids removed
		per cent		*per cent*		
Residential district	59.6	14.2	50.8	4.2	10	34
Average district	55.4	13.5	50.3	4.8	16	6
Factory district	53.3	10.3	48.3	5.1	24	2

TABLE 4

Females

SCHOOL	AVERAGE WEIGHT IN POUNDS	VARIABILITY	AVERAGE STATURE IN INCHES	VARIABILITY	PERCENTAGE OF CHILDREN HAVING	
					Infected adenoids	Adenoids removed
		per cent		*per cent*		
Residential district	58.0	12.1	50.8	4.1	0	12
Average district	53.5	13.4	49.8	4.1	14	2
Factory district	51.7	11.7	48.5	4.5	10	2

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[2] As may be readily computed from the data tabulated above, the "standard deviation" or *absolute* as distinguished from *percentage* variability of stature shows tendency to attain a maximum at 14 years in the boys and at 11 to 12 years in girls. This coincides with results previously obtained by F. Boas, Report of the Commissioner of Education, Canada, 2 (1896–97), p. 1541.

way of indication of the degree of medical care received by the children of the three classes the percentage of cases of infected or operated adenoids is included in the tables.[3]

From these results it is evident that increasing unfavorability of environment results in a parallel increase of deficiency in weight and stature. It is a strikingly significant fact, also, that as the favorability of the environment decreases the proportion of medical care extended to the children, as indicated by the percentage of removed adenoids, also decreases, while the degree of medical neglect, as indicated by the percentage of infected and unoperated adenoids, undergoes a parallel increase.

The variability of weight, which is normally high, decreases in an unfavorable environment in consequence, as explained above, of the retardation of the rate of growth in weight which is also an effect of the unfavorable environment, while the variability of stature, which is normally much less variable, increases in consequence of the enhancement of normal deviations from the average which in this case more than compensates for the reduction of variability due to slackening of growth. We may infer that among children of this age a defect in stature and weight, with a high variability of stature and low variability of weight is indicative of the operation of an unfavorable environment.

SUMMARY

1. The rate of growth in weight of school children of both sexes increases from the seventh to the fifteenth year and variability in weight undergoes a parallel increase during this period. There is a decided tendency for maxima of variability to coincide with maxima of yearly increment in weight.

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[3] The parents of the children attending the school situated in the industrial district included a large proportion of individuals of foreign birth. It is not believed, however, that this factor invalidates the conclusions drawn from the above comparison, for on the one hand, there is no reason to suppose that the children attending the other schools were less heterogeneous racially than the children attending the school in the industrial district, for they represent in the majority of cases the offspring of persons whose parents were themselves of non-American nativity, and on the other hand, while racial heterogeniety in the absence of other factors might be expected to enhance the variability of *every* characteristic and dimension, the children from the industrial district were actually *less* variable in weight although more variable in stature than those from the average and residential districts.

2. The stature of school children during this period increases at an almost uniform rate and the variability of stature is correspondingly uniform.

3. The variability of stature is much less than the variability of weight, from which we may infer that as a criterion of abnormality the measure of stature is more reliable than that of weight, while as a sensitive indicator of the effects of environmental, physiological or dietetic fluctuations, provided statistical methods of investigation are employed, the measure of weight is to be preferred to that of stature.

4. Among children of 8 years of age it is found that increasing unfavorability of environment and lack of medical care results in a parallel increase in deficiency of weight and stature, accompanied by a decrease in the variability of weight and an increase in the variability of stature.

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CONTRIBUTION TO THE PHYSIOLOGY OF THE STOMACH

XXXIX. The Hunger Mechanism of the Pigeon and its Relation to the Central Nervous System

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Some time ago Professor Carlson suggested that the restlessness of decerebrate animals might be correlated with the motor activities of the digestive tract. At the same time such preparations would serve as test objects for studying the relation of the gastric activities to the cerebral or other centers. Two lines of experiments have been carried out in studying these two questions.

METHODS

Study of the changes in gastric activities was carried on by the method for graphic registration described by Professor Carlson (1) and by direct observation. The two methods have been employed as checks against each other in the effort to determine the advantages of each and the errors to which each is subject. Tracing No. 1 was made by holding the bird quietly in the hand and indicating the occurrence of contractions with a signal magnet writing against a moving drum.

The gastric apparatus of the pigeon is anatomically divided into three parts: crop, proventriculus or glandular stomach, and the muscular stomach or gizzard. The crop is described as a simple dilation of the esophagus and has a similar structure. [Details are given by Gadow in Bronn's Manual (2)]. The movements of the crop may be directly observed by plucking the feathers from the lower end of the neck and the upper part of the breast. To record these movements a small rubber balloon may be passed down the esophagus, but since this leads to persistent swallowing movements a better way is to make a fistula through which the balloon may be introduced, without inconvenience or discomfort to the bird. Since in pigeons it is unnecessary to take elaborate aseptic precautions for surgical experiments all that need be done is to etherize the bird and make an incision through the skin and muscularis of the crop and insert a small piece of rubber tubing to keep the fistula open. If this tubing be removed the fistula will close in two or three days and the bird is none the worse for the operation. Two or three fistulae may be made in the same animal if needed. Direct observation of birds with and without such fistulae show that the presence of the small opening does not modify, or only very slightly, the normal movements of the crop.

Tracings of the movements of the proventriculus and gizzard in the normal bird may be obtained by pushing the balloon into the gizzard through a fistula made in the midline of the crop (fig. 2).

By the term decerebration in this paper is meant the removal of all the forebrain anterior to the thalamus. The technique of decerebration was developed by the trial-and-error method of experience. Practice showed that the principal difficulty lay in removing all of the forebrain without injury to the thalamus and cerebellum and without irremedially injuring the cerebral circulation. The layer of supraventricular cortex is very thin and that part of it which, in its position, corresponds to the occipital cortex of mammals is very easily overlooked until revealed at post mortem. Scrader (3) and Munk (4) state that about four-fifths of their completely decerebrated birds died shortly after the operation. In the present series of experiments, out of forty attempts only six birds satisfied the requirements of recovery from the "shock" of the operation, exhibition of typical decerebrate behavior, and post mortem confirmation of complete decerebration without trauma of the remaining parts of the brain. The technique finally adopted was as follows: In the etherized bird the cranium was exposed by a longitudinal incision of the skin. The bone overlying the cerebral hemispheres was then carefully removed, except that part directly over the median sinus. The dura should not be torn during this process. Then with fine pointed scissors the dura was laid open by the incision near and parallel with the medial and transverse sinuses. This was done for both hemispheres. All of this can be done without bleeding. A probe with a point curved to fit the posterior border of the brain was then inserted under the dura and the brain substance removed while the dura was left intact. Hemorrhage was controlled by gently packing with cotton. When the greater part of the bleeding had ceased, the cotton was removed, the cranial cavity left empty, and the skin was sutured over the bridge of bone that had been left in position. In successful preparations, after death it will be seen that the edges of the dura have coalesced to form a sac filled with clear cerebrospinal fluid. All traces of hemorrhage have disappeared and the circulation of the remaining parts of the brain is as nearly normal as is possible after such an operation. Any postoperative bleeding is allowed to take care of itself. No dressing was applied in order to avoid pressure on the remaining parts of the brain.

LITERATURE

Descriptions and discussions of the behavior of decerebrate animals and bibliographies are given in all the better text- and handbooks. [See especially Schrader (3) and Tschermak in Nagel's Handbuch (5)]. Attention however must be called to the following points, significant for the present work.

Rolando in 1809 and Flourens (6) in 1822 first showed that decerebrated pigeons could be kept alive for long intervals of time.

Longet (7) showed that the behavior of such birds depended on whether or not the thalamus was included in the decerebration: that birds with the thalamus intact exhibited spontaneous movements, while those with the thalamus destroyed did not.

In striking contrast to the long continued discussions of the psychic or mental capacities of decerebrate animals, Vulpian (8) maintained that the "spontaneous movements are purely mechanical." "There is lacking in their reputed voluntary movements that capricious spontaneity that is seen in the normal animal." "Apres l'ablation du cerveau au contraire ces mouvements sont dus a des excitations, soit exterieures . . . . soit interieures et d'un certain ordre telles que la fatigue, la gene, que determine une meme attitude trop longtemps prolongee," etc. But he did not include visceral influences as a factor.

Schrader studied the influence of light, sound, and other external stimuli on these spontaneous movements and stated that hunger and thirst were factors. " . . . . bei den automatischen Bewegungen die Erregungen den vegetativen Organen enstammen . . . . die Bewegungen unseren Tauben von manchen vegetativen Reizen beeinflusst werden." But no objective proof of changes in the viscera was given.

Munk states specifically that the completely decerebrated bird with the thalamus intact moved about very little "so lange der Kropf nicht leer oder nahezu leer war. Aber war den Kropf leer geworden so erfolgten jene ersteren Bewegungen (stretching, preening, etc.) haufiger und auch die Gehbewegungen wiederholten sich desto rascher, je langer die neue Futterung unterblieb."

Goltz's (9) famous decerebrate dog was constantly restless during the day but quiet at night.

With reference to the activities of the gastric mechanism, Brown Sequard (10) noticed that rhythmic movements of the crop may be seen. Since then many physiologists have studied the intrinsic factors and the external nervous control of these organs. (Claude Bernard (11), Mangold (12), Stubel (13), Magnan, (14), Doyon (15).

Rossi (16) found that in the crop (L'ingluvie) two types of contractions occur: Periodic peristaltic contractions of the upper part and rapidly repeated rhythmic contractions of the lower part of the crop. These were recorded by the balloon method. He states that these contractions are not present when the crop is empty.

Doyon recorded similar contraction of crop by the balloon method. He noticed an alternation of the contractions occurring in the glandular stomach with those of the muscular stomach: that if the bird is hungry the crop is at rest, but distention of the balloon causes contractions. Also, that the crop is innervated by the vagus and electrical stimulation of the nerve causes a tonic contraction of the crop and rhythmic contractions of the gizzard.

Finally Kato (17) in a recent paper from Mangold's laboratory gives measurements showing that the pressure exerted by the contractions of the gizzard during hunger are greater than those occurring after feeding.

RESULTS

In this report the results stated for decerebrate birds are from birds which have been kept for one to four months after decerebration. For a few days after decerebration there may be a period of shock marked by vascular, temperature, and gastro-intestinal disturbances. These do not always appear, and, as pointed out by early workers, if they do not the "prognosis" is good.

Normal birds. When a relatively large balloon is put in the empty crop and then distended, the bird will frequently set up a shrugging sidewise movement that indicates that the balloon is a source of irritation. It shows very clearly that stimuli from the crop can induce changes in the behavior of the bird. This irritating action can always be produced by sufficient distension of the balloon. It is sometimes

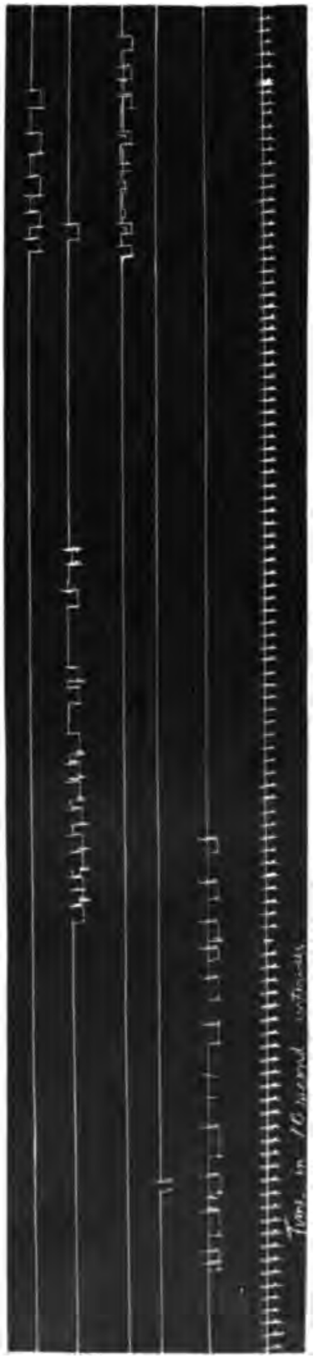

Fig. 1. Decerebrate bird, second day of hunger. Beginning of contraction of crop indicated by down stroke of writing point; end of contraction by up stroke. Time in 10 second intervals.

seen after overdistension of the crop with food. These head and neck movements are of course not to be confused with contractions of the crop which may or may not be present at the same time. Avoiding these effects of overdistension there occur two types of contractions of the empty or nearly empty crop. First, strong peristaltic waves that run over either the entire crop, if it be empty, or only part of it, according to the degree of distension with food. Second, much more rapid rhythmical contractions at the extreme lower part of the crop. Both types are visible in the empty crop.

The frequency of the peristaltic waves and their point of origin varies with the volume of the contents of the crop. After feeding they seem to be entirely absent for a short time (30 to 45 minutes). Then at intervals there occur one or two contractions which can be detected by the balloon. In an hour or two there appear groups of three or four of these waves, and they gradually increase in frequency and vigor so that after five or six hours there may occur at intervals of a few minutes contractions in groups of six to twelve or more. By this time the bird is seeking food but if no food is supplied and the crop is allowed to empty, this same peristaltic or tonic activity continues. Sometimes the contractions are rapidly and continuously repeated for several hours, but they usually occur in groups periodically (fig. 3). Efforts to determine whether or not there was any change in the character of the contractions according to the duration of the starvation period showed that sometimes as the hunger period is prolonged (two or three days) continuously occurring contractions appear, at other times they do not. These variations occur in the same bird at different times.

To obtain tracings it was found necessary to keep the birds in darkened cages to keep them quiet. If while a period of contractions is occurring, a light be suddenly thrown on the bird the contractions cease immediately (fig. 4). A sudden noise will have the same effect. Attempts to capture and hold the bird in the hand at once inhibit the contractions. Spontaneous struggling of the bird of course does the same.

The contractions of the empty crop in the normal bird are not easy to demonstrate except by the balloon method. For the normal hungry pigeon in a cage is usually in a state of restless excitement, and it can be seen only that the crop is empty. But if such a bird be quieted in a partially darkened cage with the observers sitting quietly near, the contractions of the crop can be seen without any form of registering apparatus.

Probably one reason for the statement of other observers that the empty crop is quiescent is the failure to control these inhibitory influences. In a blinded pigeon but with the cerebrum intact it is much easier to observe these changes in the crop. The blind bird usually but not invariably sits quietly in its cage except when at intervals it is driven by hunger or thirst to hunt for food and water. In such a bird patience alone is required to discover that if the bird is quiet and the crop empty, at intervals one or more deep peristaltic waves will run over the entire crop and this is frequently the precursor of the bird's shifting its position or walking about the cage.

At other times instead of periodic contractions the entire crop may be so closely constricted as to nearly obliterate its lumen. It is evident that according to the explanation given by Cannon (18) of the relation between tonus and distension, peristaltic contractions will not appear on such a constricted organ. Between this condition of contraction of the whole organ and that of a partially relaxed crop over which run deep peristaltic waves, there may be found all intermediate gradations in the same bird at different times.

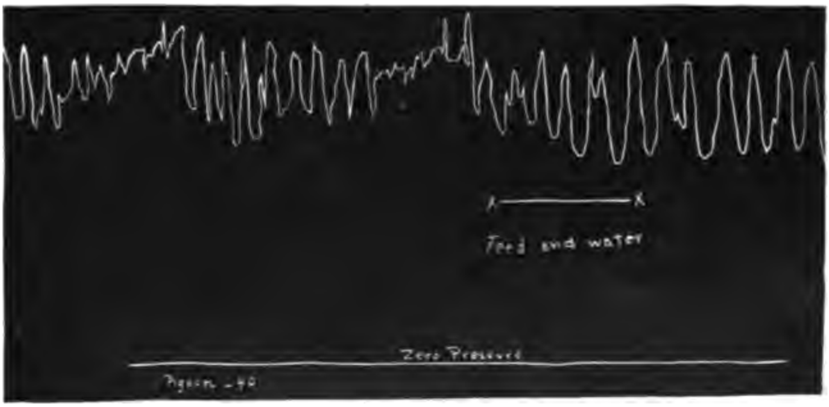

FIG. 2. Decerebrate bird, 24 hours hunger. Balloon in the gizzard.

The rapidly repeated rhythmic contractions of the lower part of the crop occur at the rate of eight to ten per minute. Each of the much slower peristaltic waves of the empty crop requires ten to twenty seconds to complete its cycle. (Fig. 5.)

Very small amounts of water and food first stimulate and then inhibit the contractions. No inhibition by taste stimuli was found.

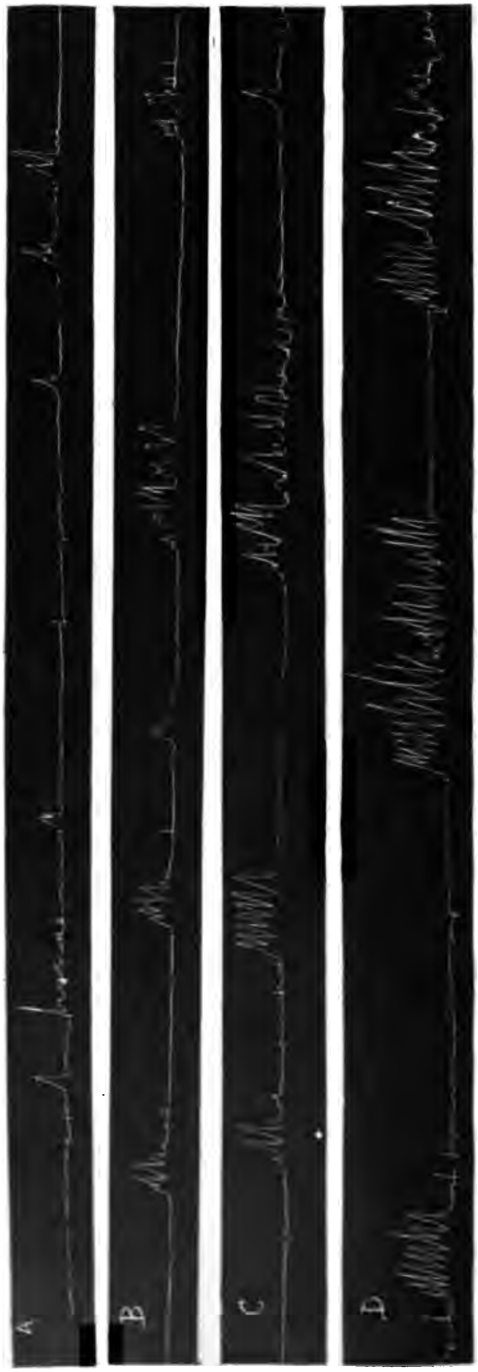

FIG. 3. Decerebrate bird. Contractions of crop. Parts of a continuous series. *a*, ½ hour after feeding. *b*, 2 hours after feeding. *c*, 4 hours after feeding. *d*, crop empty 18 hours after feeding.

This is not surprising since these birds are dry-grain feeders. Lesions of the cerebellum or unilateral destruction of the semi-circular canals leads to a rather striking inhibition of the crop activities and hence stasis of food. This effect is more marked in birds with the cerebrum intact than in decerebrate birds, but it is true of both. The analogy to vertigo and seasickness in man with the accompanying gastric disturbances is obvious. Simple prolonged anesthesia or hemorrhage from the cranial arteries or sinuses does not have this effect.

Decerebrate birds. The preceding facts which with patience can be made out in the normal bird are more easily demonstrated in the decerebrate animal, for here inhibitory influences are at a minimum. They may be studied either by direct observation or by the balloon and graphic tracing method. As Doyon states, these contractions may be induced by distension with the balloon. But the balloon may be discarded and the still simpler device adopted of so pressing on one part of the crop with the fingers as to distend the remainder with the contained air. This recalls Auer's observation that in the nearly empty stomach of the rabbit distension with air induces lively contractions. But by either method, the response of the crop is periodic; it can not always be induced by simple distension but only at intervals. Evidently there is a periodic alteration of tone or of ability of the muscle to respond to the stimulus of distension.

These contractions occur either singly or in groups of two to twelve at intervals of ten to sixty minutes. At times they may run on continuously for an hour or more. Both types of grouping have been noticed in the same bird at different times. These contractions which have been spoken of as "spontaneous" by other workers might be designated the hunger contractions of the bird, for their appearance usually means subsequent restlessness of the bird.

One of the easiest ways of visibly demonstrating these contractions is to take a bird whose crop is empty and the bird restless and give it plenty of water. The crop relaxes, and the bird assumes the typical sleeping position. The crop slowly empties itself: its tonicity increases and when the bird again begins his wanderings deep contractions can be seen to be running over the incompletely constricted crop. Their occurrence can be understood as the resultant of that reaction between tone and distension which has been described by Cannon for the mammalian stomach.

If a very small amount of food or water is given this sets up a lively play of contractions in the crop. These soon disappear and there

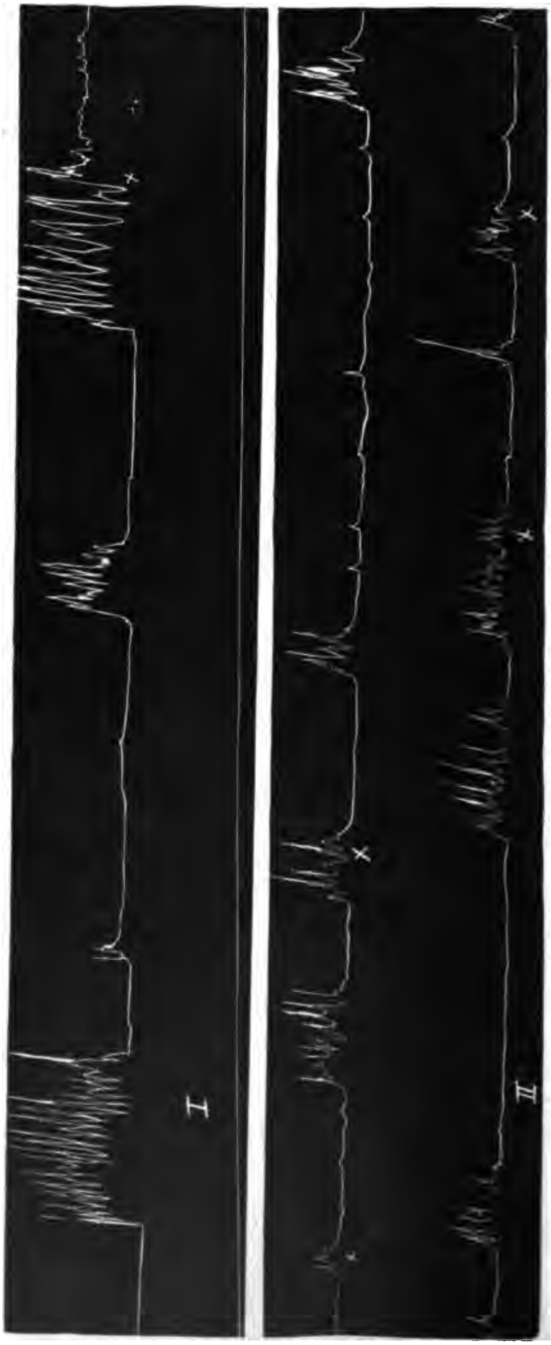

Fig. 4. Normal bird with fistula in crop. 24 hours starvation. I. At *x* bird was taken from cage by hand. II. Bird in a covered cage. At *x* cover lifted and light thrown on the bird. Time: length of each tracing is 20 minutes.

follows a marked relaxation of the previously constricted or contracting crop. This relaxation is considerably more than is necessary to barely permit the reception of the food swallowed. Solid food is a more efficient relaxing agent than an equal volume of water. With water there is some quantitative relation between the volume of fluid introduced and the relaxation of tone. Thus two to four cubic centimeters of water does not always have this inhibitory action, while eight to twelve cubic centimeters is followed by relaxation.

In the decerebrate bird which has only recently recovered from the shock of operation, light, noise, and handling the bird, have no inhibitory effect on the contractions. A month or two later, light and noise have no inhibitory effect, but handling the bird frequently stops the contractions. The reaction to food and water is identical with that of the normal bird. Lesions of the equilibratory apparatus have an inhibitory effect on the crop activities.

With reference to the motor activities of the proventriculus and gizzard tracings have been made which (in so far as the balloon method is justifiable) show that both of these parts of the hungry bird are vigorously and continuously contracting. As is shown by the tracing (fig. 2) periods of tetanic contractions occur.

With the thalamus intact, the conclusion seems unavoidable that when the hemispheres are gone, inhibitory reactions to external influences are gone but are still retained for intrinsic stimuli. So far as the gastric mechanism is concerned loss of the hemispheres means only that the gastric activities are no longer related to distant influences.

How do these factors relate to decerebrate restlessness? This restlessness is simply a continuous or periodic walking about. It is a familiar fact that in the dog after transection of the spinal cord, the stepping reflex may be easily elicited. The writer was somewhat surprised to find that in the dog after removal of the cerebral cortex with the thalamus left intact, any painful stimulus produced coördinated walking movements of all four legs (Sherrington, 19). Hence it seems fair to assume that in the bird decerebrate restlessness is only a reflex response to some stimulus. In such a bird, light and sound may be influencing factors but certainly are not the essential or adequate stimuli, for the blinded, decerebrate bird shows the same type of restless wandering as one with the eyes intact.

Moreover, whether or not in the decerebrate pigeon the restlessness is continuous or periodic with few exceptions depends on the size of the cage. A hungry bird which tends to wander around continuously

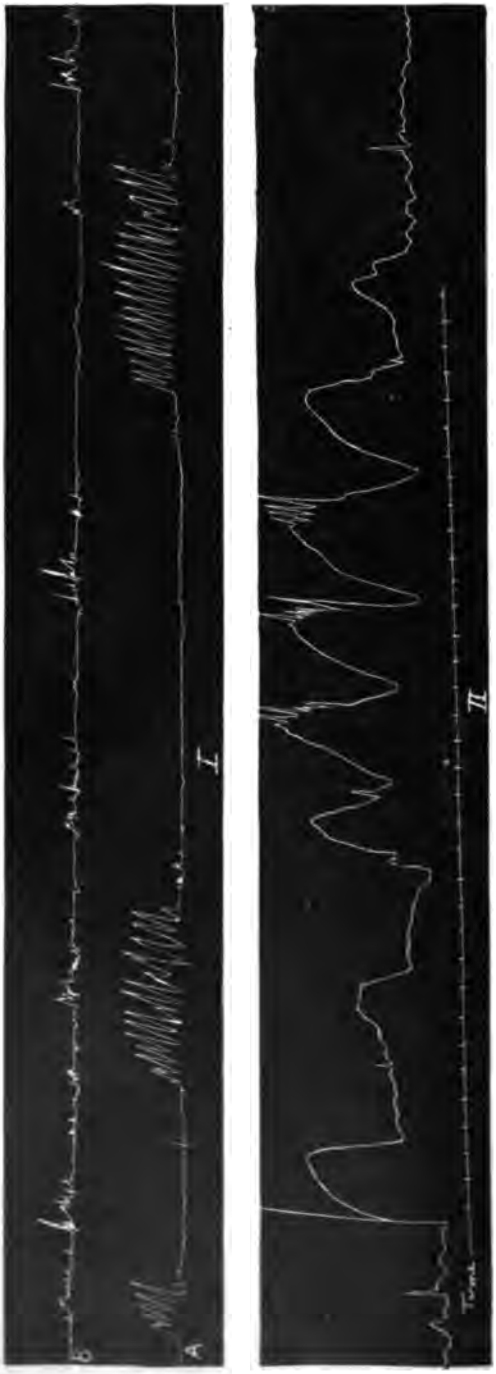

FIG. 5. Decerebrate bird. 24 hours since last feeding. In I, *a*, contractions of crop before feeding. *b*, just after feeding. In II, 24 hours hunger. Same as above except faster drum to show time interval of each contraction. Time in 5 second intervals.

in a large open space will on the same day react periodically in a smaller cage (20 by 30 inches). The hungry (not thirsty) bird exhibits periodic activity of the crop and periodic restlessness. The contractions of the crop are not caused by the restlessness of the bird for they run their course with the bird held quietly in the hand. And it has been repeatedly seen in the decerebrate birds and in blinded pigeons with brain intact, that the crop activity begins before the restlessness. Of course once started the restlessness tends to continue after the exciting stimulus has disappeared and may continue until fatigue sets in or until the bird is stopped by an obstruction. Cessation of the walking occurs only if the exciting stimulus is no longer present.

But the changes in the crop are not the only possible exciting factors, for it can be shown by feeding dry corn that the bird is subsequently restless and this can be ended by giving water. Furthermore, with plenty of food and water birds sometimes walk around for a short time, defecation follows and the bird becomes quiet. Evidently three possible stimuli inducing the walking reflex are the visceral impulses associated with thirst, hunger; and defecation. The last two are connected with hypermotility of the digestive tract. Conversely, by keeping the birds well fed and watered they seldom move about and in these occasional cases the movements can be understood as responses to fatigue or cutaneous stimuli.

If a normal pigeon is etherized and the viscera exposed the gizzard will be seen to be quiet, the intestine may or may not exhibit peristaltic contractions. If such a bird has previously received an intraperitoneal injection of one cubic centimeter of 0.1% pilocarpine sulphate the gizzard and intestine will be seen to be contracting vigorously. If the same dose be given to a decerebrate bird which has previously been fed and watered, in a few minutes the bird will either begin walking around or executing a rather comical stepping reflex without actually moving its position—a sort of "mark time" reflex, as it were. One-fourth cubic centimeter of pituitary solution (Armour) produces similar effects. Five-tenths to one cubic centimeter of 0.1% atropine abolishes the pilocarpine restlessness and likewise inhibits the contractions of the gizzard. The converse experiment may also be carried out. The decerebrate bird is given water but no food. While exhibiting the restlessnesss characteristic of hunger an injection of atropine will abolish the walking and the bird relapses into a somnolent position. This is suggestive but of course not a conclusive line of experimentation. Drugs which stimulate the visceral activities cause restlessness in the bird. Drugs which quiet the viscera quiet the restlessness. Possibly stimulation of the central nervous system by the drugs might account for the changes in the response. But no stimulation for the central nervous system has been described for pituitrin and it produces visceral and reflex effects similar to pilocarpine. Atropine, if anything, is a stimulant of the central nervous system and yet it abolishes the walking response while at the same time quieting the viscera. Other drugs less powerful in action give varying results. Simple laxatives such as lactose and excess of water frequently cause restlessness with defecation but not always. These substances however do not cause increased tonus of the gut.

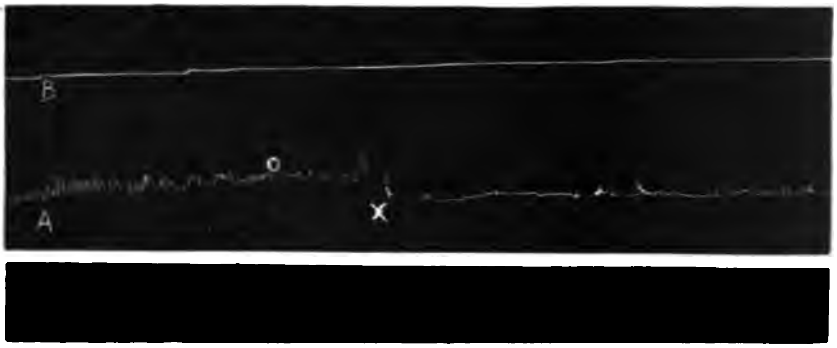

Fig. 6. Decerebrate bird, 24 hours after feeding. Balloon in lowest part of the crop. At *x*, water given by mouth. *b* is continuation of *a*. Time in 6 second intervals.

All of this suggests that two factors remain which if possible to control or estimate might reduce the behavior of the decerebrate bird to mechanical terms. These two factors are; threshold values for visceral stimuli and variations in the irritability of the central nervous system. It seems to the writer that if it is possible to reduce the study of visceral impulses to a quantitative basis the decerebrate animal is par excellence the test object for such work.

With reference to the reflex irritability of the central nervous system prolonged observation convinces one that variations do occur, although so far as the writer knows little has been done on the question. Further work is planned on this but the following may be noted here:

A hungry bird, not fed for several days, is restless during the day and quiet at night. This is not a question of illumination only, for the blinded decerebrate bird acts in the same way. However, this is not an invariable rule, for I have seen such birds periodically restless during the night and have seen these periods run parallel with periods of crop activities. But as a rule the bird is quiet at night in spite of changes in the digestive system. I have also seen that a louder noise is necessary to cause a reflex lowering of the feathers or opening of the eyelids when the bird has been fed than when it is hungry. The variations in irritability change so markedly as time is allowed for recovery from shock that a much more careful study of this will have to be made.

SUMMARY

In normal and decerebrate pigeons the crop when nearly empty and during subsequent starvation exhibits hypermotility or hypertonicity which is usually periodic but may at times run continuously for several hours.

In the normal bird this ~~hyper~~activity is inhibited by food, by water, by any conditions causing fear or surprise, or by labyrinthine and cerebellar lesions.

In the decerebrate bird (thalamus intact) water and food inhibit the hyperactivity. Inhibitory reactions to non-painful external stimuli are wanting but persist for intrinsic painful stimuli or lesions of the equilibratory apparatus.

Three kinds of visceral influences normally stimulate the restlessness of the decerebrate bird: Hunger associated with hypermotility of the gastric mechanism; thirst; and sometimes intestinal impulses. Pilocarpine and pituitrin augment visceral tone and contractions and induce the walking reflex; atropine quiets the viscera and abolishes the restlessness.

Variations in reflex irritability occur in the well kept decerebrate bird.

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SOME UNUSUAL EXPERIMENTAL LESIONS OF THE CEREBELLUM AND MEDULLA OBLONGATA

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In the course of some experiments on the semicircular canals and the otic labyrinth, a number of lesions of the cerebellum and the medulla oblongata resulted, either from slips in the experimental technique or from accident. They may be regarded as experiments contributing much to our own education while we were developing our operative methods. But as the results did not bear directly on the question of the effects of stimulation or extirpation of the labyrinth, they were not included in our first paper on the labyrinth (1). There is but a single lesion of each kind, except in the case of rupture of the posterior meningeal artery, but we present them at this time in the hope that some of the results may not be without interest to others who may have had similar accidents, and none of them are without a distinct interest to students of the physiology of the central nervous system. Some of the lesions were not such as one would care to duplicate more than necessary, and one or two, being the result of accident, might not be easily duplicated. The third one, on the lesion of the flocculus cerebelli, illustrates particularly well the statement, due, we believe, to Hitzig, that slight lesions may sometimes be more instructive than graver ones.

I. DEATH FROM HEMORRHAGE FOLLOWING INTRACRANIAL RUPTURE OF THE POSTERIOR MENINGEAL ARTERY

In the course of some experiments on section of the vestibular nerve intracranially, intracranial rupture of the posterior meningeal artery was followed by death in less than twenty-four hours. It had been observed in previous experiments that extracranial rupture of the artery had no noticeable effect. The method followed in sectioning the vestibular nerve was to make a small trephine opening in the occipital bone over the medial edge of the petrous portion of the temporal bone and a little above the foramen of the posterior meningeal artery. As soon as the trephine had cut through the thinner part of the occipital bone at the medial edge of the temporal, the button of bone was removed and a probe inserted through the small opening in the occipital bone. The probe was passed downward and forward between the skull and the dura in the direction of the internal auditory meatus. In some experiments, the internal auditory meatus was not reached and the vestibular nerve not injured, as shown at post mortem examination. But irrespective of the success or failure of dividing the vestibular nerve, some of the animals were found dead the next morning. The immediate post-operative results were never more severe than in those experiments in which the labyrinth had been opened from the exterior, without disturbing the intracranial structures in any way. It would have been easy to attribute the deaths of our animals to shock, but our suspicions had already been aroused with reference to shock (2) as a cause of death and an error in procedure seemed much more plausible. Post mortem examination showed in each case, an extensive hemorrhage outside of the dura, never extending anterior to the tentorium, usually not extending beyond the median line of the skull, but extending downward and backward along the medulla oblongata. It is our opinion that death resulted from the pressure on the medulla due to the hemorrhage from the ruptured artery. Avoidance of the posterior meningeal artery or ligating it outside of the skull ended the trouble.

The death of the dogs seems to us to be of interest in connection with death from accidental fracture of the base of the skull in the human. Inquiry has failed to elicit any information on intracranial rupture of the posterior meningeal artery in these cases, several pathologists and surgeons saying that they never had seen a case. It would seem probable, however, that this artery is sometimes involved in the fracture, and its ligation, or the relief of pressure over the cerebellum and medulla by removal of the bone about the foramen magnum might afford some prospect of relief in a small percentage of cases of what is now a hopeless surgical condition. It is not to be expected that damage to the nervous tissue from internal hemorrhage will be very much decreased by operative procedures, but the increased intracranial pressure, which may in itself be a sufficient cause of death, would be relieved.

II. A FOREIGN BODY IN THE LEFT LATERAL LOBE OF THE CEREBELLUM

In one of these experiments, in exploring with a probe the operation wound in the bone resulting from removal of the labyrinth, the point of the probe slipped through into the cerebellar cavity and broke off. The condensed protocol of this experiment follows:

January 30, 1910. Black and tan dog. Removed left semi-circular canals. Point of dissecting probe broken off in bone and could not be removed.

After operation marked nystagmus in both eyes; appeared first in left eye.

February 6. Nystagmus disappeared on February 4. Today note:

1. Marked inclination of head to left side.
2. Cork-screw twist of head in cervical region.
3. If dog is lifted up, has marked tendency to fall to left.
4. When walking, dog inclines to left, and tends to go in small circles.
5. Drop reflex little affected.
6. Goes upstairs but refuses to come down; if induced to do so immediately falls over to left; and in one attempt rolled down the whole length of the stairs.

Dog killed.

Post-mortem. Piece of broken off probe was found imbedded in the left lobe of the cerebellum. No hemorrhage and no adhesion of dura mater to the cerebellum.

Fixed in Müller, with bone attached on left side.

The presence of the foreign body in the left lateral lobe of the cerebellum did not modify the type of nystagmus, nor appreciably prolong its course. Walking in a circle and falling over toward the side of the injury probably persisted slightly longer than they would after a purely labyrinthine lesion on one side.

III. LESION OF THE LEFT FLOCCULUS CEREBELLI WITHOUT INJURY TO THE VESTIBULAR NERVE

A slight lesion of the left flocculus cerebelli, without involvement of the eighth cranial nerve, did not cause nystagmus, torsion of the head nor rolling over to one side. The dog lay on the left side with left fore and hind legs extended and right legs often drawn up to the body. The head was outstretched and the nose on the floor. When placed on the right side, the dog attempted to stand up and fell over to the left.

The left eye was deviated outward and the left nictitating membrane was drawn to the median line. Oscillations of this eye occurred, but no constant movement could be made out. The right eye was not deviated and the right nictitating membrane was not more prominent than usual.

The dog became playful and was able to go up and down stairs, and to run about on icy walks, or to jump over his lead rope without any great difficulty, at the end of six weeks.

At the end of two months the dog was killed. A study of his reactions at this time showed that he still had a slight tendency to fall to the left. Often, when the dog did not fall completely over, he leaned to the left and the right hind foot was raised from the floor. The left forefoot swung inward in walking, particularly at the moment when the foot was raised from the floor at the beginning of a step. In starting to walk, he often went to the left before becoming able to go in a straight line. The drop reflex was manifested first in the left hind leg, but the right hind leg came down under him in a way better to break the fall. In walking, the left hind foot was out to the left, and the right hind foot nearly behind the left fore foot. There was some slight oscillation of the eyes on looking to the right, but it was inconstant. The eyes were more readily deviated to the right than to the left, but there was no noticeable nystagmus. The left eye was deviated a little to the left when the dog looked straight ahead.

The undoubtedly greater persistence of disturbances of locomotion and equilibrium, and the more enduring deviation of the eye, together with the fact that the homolateral eye alone was deviated, differentiate this lesion from a labyrinthine lesion. The fact that this dog lay preferably on the injured side is in direct contrast to the fact that a dog with a more extensive lesion, involving the lateral half of the cerebellum, lay preferably on the uninjured side.

The protocol of the experiment follows:

November 15, 1909. Young fox terrier dog. Operation on *left side.*

Trephine wound made lower down nearer to condyle and to lateral side of condyle on *left side.* Large vein opened and very considerable hemorrhage, stopped by removing bone and inserting cotton and exerting pressure.

The second trephine wound was made higher up. Through this the probe was inserted till the seventh nerve was found and then section was made. When the seventh was touched there was distinct contraction of the face muscles.

As the dog began to come out of ether there was noted (1) Twitching of the angle of the mouth on the left side, none on the right. (2) The nictitating membrane on the left side was drawn to the middle line; right side normal. (3) Corneal reflex marked in the right, very little in the left eye. No nystagmus observed.

November 15, 2 p.m. Dog found lying on left side in cage. Had moved some since he was first placed in the cage, but only a little. Made no sound when cage was approached and door opened. Wagged tail when petted and spoken to.

Nystagmus very doubtful; there was possibly a slow horizontal nystagmus in right eye, but it is inconstant and more probably due to voluntary movements of the eye. Right eye is not deviated and right nictitating membrane is not more prominent than usual.

Left eye was deviated outwards and the nictitating membrane was drawn nearly to the median line of the eye. No constant movement could be made out in this eye.

The dog lay on his left side with left fore and hind legs extended, the fore leg more extended than the hind. Head outstretched with nose on the floor. Right fore foot usually drawn up and often right hind foot also. When placed on the right side, the dog tried to assume the erect position and then rolled over on the left side. Repeated. Occasionally left fore foot was stretched out as in decerebrate rigidity.

Right legs (fore and hind) were sometimes drawn up in inspiration, and tremors might occur toward the end of inspiration. Tremors became much more marked after the dog had lain uncovered for awhile, and might persist throughout expiration, though with diminished intensity.

The corner of the mouth on the left side did not gape.

November 21, 1909. Dog able to stand alone for a little time and to walk a short distance, but fell over to the left side. Left hind foot was away out to the left side in walking, and right hind foot was nearly behind left front foot.

November 28. Fell to left side. Hind limbs abducted; left eye deviated outward.

December 21. Dog demonstrated before joint meeting of the University of Chicago Biological Club and Chicago Neurological Society. Walked up and downstairs without falling, and ran over the slippery walks with ease.

January 16, 1910. Brought down for examination. In good flesh. Playful. Tendency to fall to left still present. Often, when dog did not fall completely over, he leaned to the left and the right hind foot was raised from the floor. The left fore foot swung inward in walking, particularly when the foot was lifted from the floor at the beginning of a step. In starting to walk, he often turned to the left before getting started in a straight line. When held up by the fore legs and "dropped" suddenly, the left hind leg was stretched out first and extended further out. The right hind foot came down under him better so as to be more efficient in breaking the fall.

Perhaps some slight nystagmus occurred when the eyes were directed to the right, but it was extremely slight and inconstant. One could not be certain of it. The eyes were more readily deviated to the left, but no nystagmus was observable. The left eye deviated slightly outward when the dog was looking straight ahead.

Etherized; 10 per cent formaldehyde in 0.9 per cent NaCl injected into carotid artery. Head removed, brain and upper half of spinal cord exposed and put into 10 per cent formaldehyde. There was a slight abrasion of the surface of the cerebellum along the path of the probe and some laceration of the flocculus.

IV. LESION OF THE MEDULLARY-PONTINE ANGLE ON THE LEFT SIDE

Unilateral rotatory nystagmus, loss of locomotor control, inability to stand, loss of the corneal reflex on the side of the lesion and a tendency to lie on the injured side were observed in a dog with a lesion at the medullary-pontine angle, involving the seventh, but sparing the ninth and twelfth, cranial nerves.

November 21, 1909. Fox terrier. Male. Rather large for fox-terrier.

Trephined at base of skull below occipital ridge and laterally to median line. Attempted to destroy eighth cranial nerve on *left* side. Severe hemorrhage. Cotton wool plug left in upper trephine hole. Wound sewed up and bandaged. No nystagmus observable at first, but nystagmus in the left eye was noticed about an hour after the operation was finished. The nystagmus in the left eye was rotatory, the longest diameter of the ellipse being in the vertical plane. There was no noticeable nystagmus in the right eye at this time. Given large hypodermic injection of morphine, as the animal seemed restless.

November 22, a.m. Dog lying on left side. Apparently had not struggled much, although a little cotton had been rubbed from the top of his head near the anterior edge of the bandage. Left eye deviated outward. Right eye looking straight ahead. Good corneal reflex in right eye, but apparently none in left. Did not try to have dog stand up as he seemed very weak.

November 23. Nystagmus present in left eye. Conjunctiva much injected. Eye washed out with boric acid solution.

November 25. Eye washed out again. Mouth showing symptoms of gangrene. Cornea covered with bluish white semi-opaque membrane. Lips on left side gape widely. No corneal reflex in left eye. Plug taken out of trephine hole near sinus under ether anaesthesia. Piece of iodoform gauze packing strip inserted in its place. Wound again bandaged.

November 28. Wound still open. Dog emaciated. Would lie only on left side. Paralysis of facial on left side. Unable to stand. Dog killed.

Post-mortem. Slight hemorrhage, extra dural, in cord. No hemorrhage in cerebral region. Hemorrhage in pontine and medullary region. Found large wound in medullary-pontine angle, involving facial nerve. Ninth to twelfth cranial nerves not affected.

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THE RESPONSES OF SINGLE MELANOPHORES TO ELECTRICAL STIMULATION

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PRELIMINARY

A considerable accumulation of evidence is at hand proving the melanophores of the lower vertebrates to be functionally modified smooth muscle cells. The responses of a variety of smooth muscle preparations to electrical stimuli exhibit certain characteristic peculiarities. If the melanophore is to be consigned to the category of smooth muscle, we should expect it to show the same peculiarities in its responses to electrical stimulation. In the absence of exact data concerning the reactions of single melanophores to electrical stimuli, and in the hope of confirming my previous conclusions, I have undertaken the experiments recorded in this paper. The results show that there is a close agreement between the responses of single melanophores and of typical smooth muscle preparations to faradic and galvanic stimulation. The evidence furnished by these experiments thus corroborates and amplifies the previous conclusion that the melanophore is to be considered a functionally modified type of smooth muscle cell.

PREVIOUS WORK UPON THE REACTIONS OF THE MELANOPHORES TO FARADIC STIMULATION

All the investigators (Buchholz (4), Pouchet (22), Lode (20), van Rynberk (29), Mayerhofer (21), von Frisch (30), Spaeth (26)) who have experimented with the effects of electrical stimulation in the skin of fishes agree that an induction current of sufficient duration and intensity produces a contraction of the melanophores. Such a contraction occurs whether the stimulus is applied directly to the surface of the fish or to an excised portion of the skin. Lode (l.c.) first observed the contraction of the melanin granules under the microscope in the excised fin of medium-sized trout.

The responses of amphibian melanophores to faradic stimulation have been less constant, though much of the conflicting evidence is to be ascribed to incorrect experimental premises. The earliest investigators (von Wittich (31), Harless (12), and Hering u. Hoyer (13), found that a tetanizing current produces a lightening of the skin of *Rana esculenta* which was reversible, the skin subsequently resuming its original dark color. Bimmerman (2), Êtérnod et Robert (8), Winkler (33), and Laurens (17) later made similar observations. Lister (19), Ehrmann (7) and others observed no effect from an induction current. The apparent discrepancy is not surprising since both Lister and Ehrmann considered the active phase of the melanophores to be the expansion. Their experiments were made upon light animals in which the melanophores were already contracted. With a strong tetanizing current the time required for the contraction of the melanophores in large larvae of *Amblystoma opacum* Laurens (l.c.) found to be at least fifteen minutes. Direct stimulation of small bits of excised skin gave similar results.

Faradic stimulation likewise produces a contraction in reptilian melanophores as was first shown by Bruecke (3) in the chameleon. The reaction in excised bits of skin is the same as in the intact animal. Both Bert (1) and Krukenberg (16) later verified Bruecke's observations. Keller (15), on the other hand, failed to observe any change in *Lacerta viridis* after stimulating with strong alternating currents. In the absence of any description of the experimental procedure it is impossible to judge the validity of this single negative instance.

From the foregoing briefly summarized experimental evidence, there seems to be no reasonable room for doubt that faradic stimulation produces a contraction of the melanophores of lower vertebrates. The exact nature of this contraction and its relation to other contraction phenomena has never been investigated.

EFFECTS OF FARADIC STIMULATION UPON SMOOTH MUSCLE

In the response to faradic stimulation both the latent period and the time required for a complete contraction may be said, in general, to be very much greater in smooth than in striated muscle. The effective liminal stimulus is also far greater for smooth muscle. This is strikingly illustrated by the classical experiment of Fick,[1] who found that an induced current of sufficient strength to produce a powerful tetanus in a frog's gastrocnemius or in the hand of the experimenter could pass through an adductor preparation of *Anodonta*, in the same circuit, without producing any sign of a contraction.

Wide variations occur both in latent period and contraction time in different types of smooth muscle. The following table, modified from Gruetzner, shows the range of such variations in a few examples of vertebrate smooth muscle. The figures have only an approximate value, however, since in each type of smooth muscle considerable additional variation occurs with a change in the strength and duration of the stimulus.

TYPE OF MUSCLE	LATENT PERIOD IN SECONDS	DURATION OF A SINGLE RESPONSE. (TIME OF CONTRACTION AND RELAXATION IN SECONDS)	OBSERVER
Stomach muscle of frog....	1.5–10	60–120	Ranvier Schultz Gruetzner Winkler and others
Retractor penis of dog and horse...............	0.80–4.5	15–20 Contraction 90 Relaxation	Sertoli
Detrusor vesicae of cat....	0.25	5–6 Contraction 35 Relaxation	Stewart
Membrana nictitans of cat......................	0.5	5–15	Lewandowsky
Ureter of rabbit...........	Fraction of second to 1 second	2–7	Engelmann

EXPERIMENTAL PROCEDURE

In studying the responses of the melanophores of *Fundulus* to faradic stimulation the procedure was as follows. After the fish[2] had been washed several times in tap water, distilled water and 0.1 N NaCl, or slightly modified Ringer solution,[3] a few scales were removed to 0.1 N NaCl or the modified Ringer solution, where they were left until the melanophores were widely expanded. A single scale was then transferred to a shallow depression slide or a diffusion chamber and was there held in place by means of a bit of cover slip and a touch of vaseline. The stimulus was applied through non-polarizable electrodes of the Zn-$ZnSO_4$ type. Drawn out tufts of absorbent cotton or camel's hair brushes served as contact points which were brought to lie on either side of the scale, with their tips 3 to 6 mm. apart. The most satisfactory results were obtained when the scale was covered by a piece of supported cover slip only slightly larger than the scale itself. The supports prevented mechanical stimulation by contact with the cover slip and the small size of the latter eliminated the possibility of an asphyxiation error due to an inadequate supply of oxygen. In many of the earlier experiments the scale was held in place and stimulated by means of a small, movable, two-pronged fork of flattened platinum wires. The effects of the polar products of electrolysis were largely eliminated by a strong current of NaCl or modified Ringer solution which maintained the C_{OH} far below a stimulating minimum.

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[1] Quoted from Gruetzner (11).

[2] Throughout these experiments 8.5 to 10 cm. females of *Fundulus heteroclitus* were used.

A series of preliminary trials showed that a record of the movements of the pigment granules in a single, unbranching process of a melanophore gave a fair picture of the contraction in the entire cell. All the graphs in the following pages represent the rate of movement of the most distal melanin granules in a single process of a single melanophore. Records of a series of responses in a single cell were always made by measuring the pigment migration in the same process.

RESPONSES OF THE MELANOPHORE TO FARADIC STIMULATION

Owing to the fact that the method of stimulation involves the passage of a considerable portion of the current through the mounting medium, it has proven impossible, up to the present time, to obtain a complete contraction of a melanophore as the result of a single make or break shock. It must, therefore, be borne in mind that the "single contractions" seen in the following curves are the result of a summation of make and break shocks.

A study of the responses of single melanophores to tetanizing currents of moderate intensity (fig. 1) brings out the following facts:

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[3] This modified Ringer was made up of 20 vols. 0.1 N NaCl + 1 vol. 0.1 N $CaCl_2$ + 1 vol. 0.1 N KCl.

1. The time required for a complete contraction and relaxation of the melanin granules in a process 0.1 mm. long is about of the order 90 to 120 seconds. This estimate is necessarily approximate since considerable variation occurs at the end of the relaxation phase.

2. The duration of the contraction phase is 25 to 30 seconds. Relaxation therefore requires from two to three times as long as contraction. The time relations for the contraction and relaxation in a single melanophore are thus approximately of the order seen in the case of a preparation of frog's stomach.

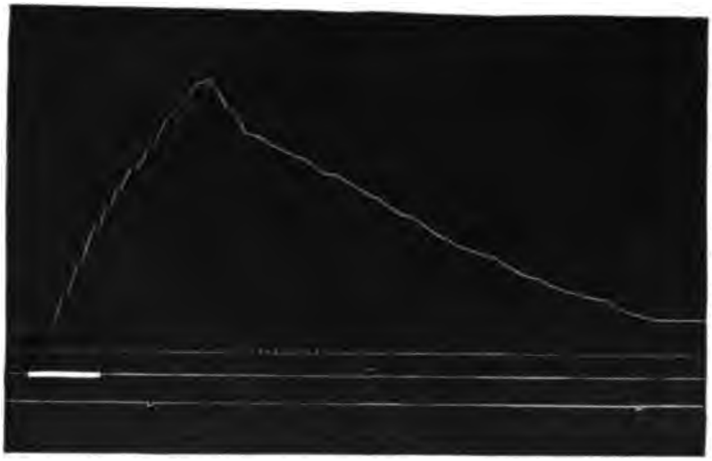

FIG. 1. Curve showing the response of a single melanophore to a brief tetanizing stimulus. The cell was removed to modified Ringer solution two hours and twenty minutes before stimulation began. The stimulus lasted nine seconds. After a latent period of 3 to 4 seconds the contraction began and lasted 21 seconds. The recovery was incomplete after 85 seconds. Upper line, time in seconds; middle line, stimulation period; lower line, time in minutes. T = ca. 22°C. The steps in the rising phase of the curve are due to the method of recording the pigment migration.

3. The steps and other irregularities which frequently appear in the curves are due to the peculiarities of the pigment migration. This migration is rarely a perfectly smooth flowing of melanin granules. The pigment frequently becomes momentarily blocked or jammed in the cell process forming a minute, opaque mass, from the proximal side of which single melanin granules, or groups and lines of granules, may be seen progressing toward the center of the cell. Such blocks

usually last from one to several seconds, though in extreme cases careful focussing will reveal several thin lines of pigment granules moving towards the center of the cell from one or more outlying blocks after the contraction is apparently completed. This phenomenon is especially likely to occur when the melanophores have been removed from the fish for several hours.

THE ORIGIN OF THE CONTRACTION WAVE AND THE LATENT PERIOD

The study of a large number of contraction curves has shown that in the response to a tetanizing current of moderate strength at room temperature there appears a characteristic latent period of 4 to 6 seconds. Other observers have recorded somewhat longer latent periods in several species of fish. Thus Lode (l.c.) found in the case of the trout that the contraction followed ½ to 1 minute after stimulating, whereas Mayerhofer (l.c.) noted a somewhat briefer latent period for *Esox*, 20 to 30 seconds, depending upon the strength of the current. In *Crenilabrus pavo* von Frisch (l.c.) found a distinct reaction in the melanophores 5 seconds after stimulating. It is significant that the latent period is in every case a long one, such as we might expect to find in a relatively sluggish tissue, like the smooth muscle of the frog's stomach.

By the latent period is meant the time that elapses between the application of the stimulus and the first sign of a contraction of pigment granules at the distal end of the process under observation. The above time relations apply only in the case of the distal pigment granules. The latent period for melanin granules lying near the proximal ends of the cell processes is less than half as great as for the distal granules. In other words the contraction wave begins at the center of the cell and spreads peripherally. The consistent difference in latent period at distal and proximal ends of the same processes in one melanophore is shown in the table below. The times were recorded on two stop watches, and different processes were selected in order to give the observations a random value.

Ex. Sp. 16–41. T. = 20°C. March 31, 1916.

9.00 a.m. Scales from a 10 cm. female to modified Ringer.

9.40 a.m. Stimulation began; 3 storage cells in series; secondary of Harvard inductorium at 6 cms., non-polarizable electrodes. Each stimulus lasted 6 to 10 seconds. Between stimuli the cell was permitted to reexpand fully. Contractions began after the following time intervals in seconds:

	Proximal		*Distal*
	2.0		5.0
	1.8		4.4
	1.8		4.4
	2.0		6.0
	2.2		4.4
	2.0		5.2
	1.6		6.2
	2.2		7.8
	1.6		4.6
	2.2		5.6
Average..........	2.0	Average............	5.4

The average distance between the proximal and distal points in the processes a which the migration first appeared was about 0.075 mm. The difference tween the average times for the beginning of the contraction at the center and the periphery of the cell—i.e., $5.4-2.0=3.4$—gives a value for the time required for the contraction wave to traverse a distance of 0.075 mm. A simple calculation shows that the speed of propagation of the contraction wave within the melanophore, under the above experimental conditions, is therefore only 22 μ per second, or 7.9 cm. per hour. This extremely slow rate seems to leave little room for doubt that we are here dealing with a passage of the stimulus through the substance of the melanophore and not along a broken branch of sympathetic nerve fiber. The origin of the contraction wave at the center of the cell suggests the possibility of the transmission of the tetanizing stimulus to the cell through a broken nerve fiber. The stimulus having reached the center of the cell, however, its further propagation appears to be independent of finer nerve fibrillae.

In the response to a tetanizing current of moderate strength, the peak of the contraction curve appears distinctly pointed, never rounded as in typical contraction curves of smooth and of striated muscle. An examination of the behavior of the melanin granules in a single process of the stimulated melanophore shows that near the end of the contraction phase a relaxation wave frequently originates at the center of the melanophore before the most distal of the pigment granules have completed their centripedal migration. At the proximal end of a process there thus appears a slowly travelling, distally moving wave of relaxation, which gradually encroaches upon and carries with it the proximally moving melanin granules. The sharp peak of the recorded contraction marks the point at which the relaxation wave has enveloped the last of the contracting pigment granules. In the

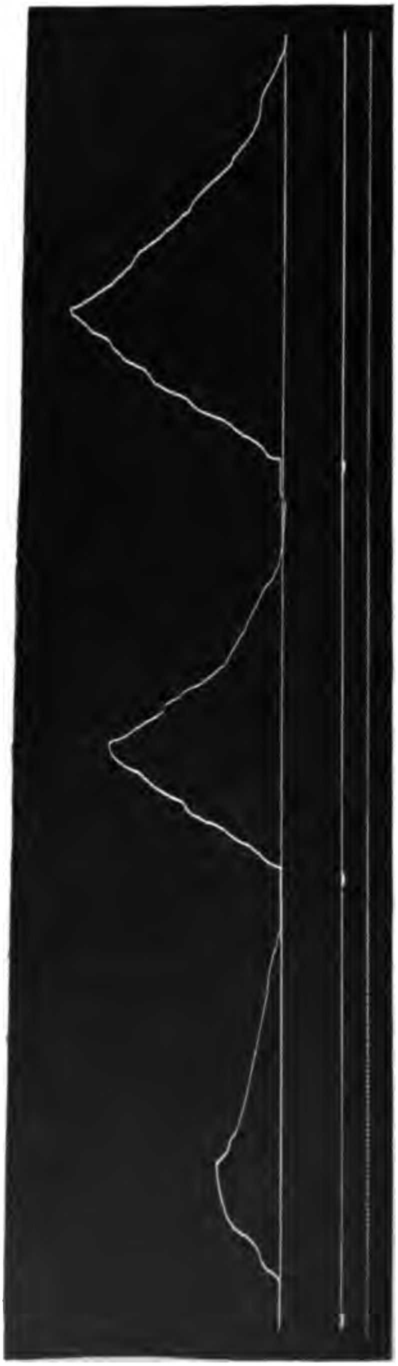

Fig. 2. Shortening of the latent period and increase in the height of the contraction following a decrease in the distance of the secondary coil from the primary. First curve 33 minutes after removal from the fish with secondary at 7 cm.; latent period, 8 to 9 seconds. Second curve 35 minutes after isolation from the fish, secondary at 5 cm.; latent period 3 to 4 seconds. Third curve 39 minutes after removal from the fish, secondary at 3 cm.; latent period 2 to 3 seconds. Time in seconds; $T = 24°C$.

melanophore we thus find that the relaxation phase may be initiated before the completion of the contraction. Thus it appears that both contraction and relaxation originate at the center of the cell, though why this should be so is not at present evident. There may exist a correlation of some sort between this central origin of contraction and relaxation waves and the centripedal direction invariably taken upon stimulation by the pigment granules.

By comparing in a number of curves the time required for a complete contraction and the actual distance traversed by the migrating pigment granules, it appears that the speed of the moving melanin granules is about 3 μ per second or 1.08 cm. per hour. This is approximately one-eighth as fast as the propagation of the contraction wave (7.9 cm. per hour).

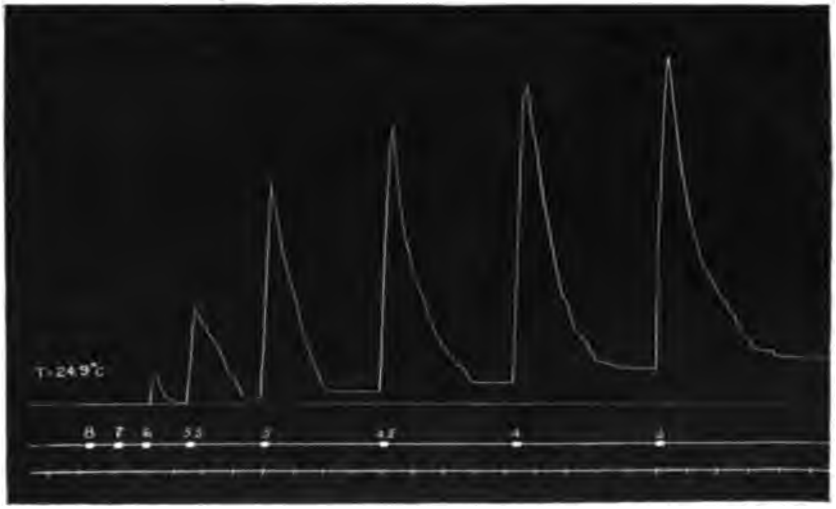

Fig. 3. Curve of increasing response with an increase in the strength of stimulus. The figures represent the distance in centimeters of secondary from primary. Stimulation began two hours and nine minutes after removing the cell from the fish. Time in minutes.

THE EFFECT OF INCREASING THE STRENGTH OF THE STIMULUS

Figures 2 and 3 show the effect of increasing the strength of the stimulating current by shortening the distance of the secondary from the primary coil. In figure 2 are shown three curves representing consecutive contractions in the same cell with the secondary at 7, 5 and 3 cm., respectively, from the primary. The stimulus lasted two seconds. The latent period becomes progressively shorter, falling from 8 to 9 seconds to 3 to 4 seconds and finally to 2 to 3 seconds. At the same time the speed and extent of the pigment contraction increases to the maximum shown in the third curve. Similar conditions obtain when a weaker stimulus is used.

Figure 3 shows the successive responses of the same cell to tetanizing currents of gradually increasing intensity. Owing to the slow rate of the drum the differences in the latent period are obscured, but the increased height of the contraction with the increase of the stimulus is very definite. These curves are strikingly similar to the series obtained by Stewart (p. 192, fig. 6, loc. cit.) upon increasing the stimulus in a preparation of cat's bladder.

SUMMATION OF CONTRACTIONS AND TETANUS

Whereas it has thus far proven impossible to obtain a complete contraction in a melanophore by a single make or break shock, by the proper spacing of a series of single break shocks[4] which are individually almost or entirely ineffective, it is possible to obtain a typical tetanus curve.

Sertoli (25) found that a single induction shock was ineffective as a stimulus for the erector penis muscle. Stewart (l.c.) obtained a complete fusion of the responses to moderate break induction currents in the cat's bladder when the interval between the successive stimuli was reduced to one second.

The summation record for the melanophore (fig. 4) shows that with a shortening of the interval between stimuli, summation appears and becomes increasingly pronounced until, with an interval of 2 to 3 seconds, complete fusion occurs. Except for the time relations, this curve is precisely like the tetanus curve obtained from smooth and striated muscle preparations.

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[4] All the experiments in this series were carried out with the help of a modified Martin key. At the suggestion of Professor E. G. Martin the current was made and broken by means of a platinum needle dipping in a vial of clean mercury covered with alcohol. The contact spark was reduced to a minimum by inserting a small telephone condenser of 1 microfarad capacity across the primary terminals.

No refractory period has been observed in the melanophore, a stimulus being effective in every phase, both of the contraction and relaxation. Stewart (l.c.) has observed a similar absence of a refractory period in preparations of cat's bladder.

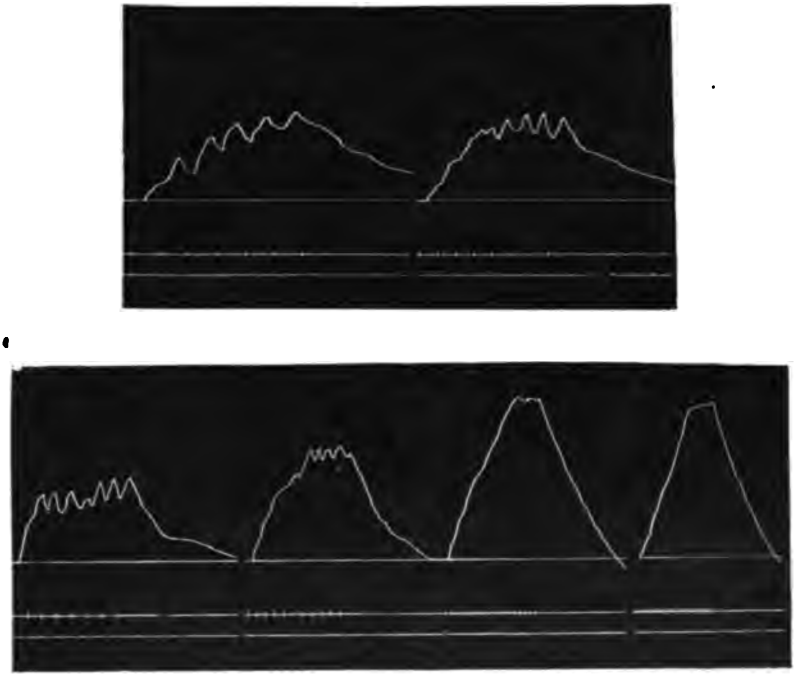

FIG. 4. Development of tetanus in a single melanophore. Powerful break induction shocks were applied at intervals of 15, 10, 8, 5, 3, and 2 seconds respectively. Stimulation began one hour and twenty-seven minutes after the removal of the cell from the fish. Time in seconds.

PREVIOUS WORK ON THE EFFECTS OF A CONSTANT CURRENT UPON THE MELANOPHORES

Experiments with the effects of a constant current upon melanophores are few in number and unsatisfactory in results. Lister (19) found that the melanophores of *Rana temporaria* failed to respond to galvanic stimulation. He believed that the absence of response in his experiments was only apparent and that, in reality, the chromatophores were paralyzed in the condition they happened to be in at the time of stimulation, thus eliminating the possibility either of a contraction or of an expansion. Hermann (14) could detect no color changes in frog larvae following galvanic stimulation. More recently Winkler (33) studied the effect of a galvanic current upon the melanophores of *Hyla*. A small piece of skin was transferred from the back of the animal to physiological salt solution and was then subjected to galvanic stimulation under the microscope. Under these circumstances Winkler observed an expansion of the melanophores —"schwarze Faeden" appearing from the deeper portions of the preparation. As to the reversibility of this process he states (p. 257):

> Beim Aufhoeren des galvanischen Reizes verschwinden die Faeden; es ist aber dabei nicht zu entscheiden, ob dieses Verschwinden durch ein Zurueckziehen der Fortsaetze oder durch ein Fortwandern des pigments erfolge.

Presumably the finer details of a pigment migration were not visible in Winkler's preparation.

The relaxation of amphibian melanophores in isolated bits of skin observed both by Winkler (l.c.) and Laurens (17) following galvanic stimulation is probably to be interpreted as the effect of OH ions liberated at the cathode. Laurens (l.c.) has recorded an expansion both in whole larvae and in small, excised pieces of skin of *Amblystoma opacum* when stimulated by a constant current through platinum electrodes.

He says (p. 611):

> When bits of isolated skin with contracted melanophores are thus stimulated on a glass slide with a current of from 3 to 4 m.a., and for about five minutes, the melanophores all expand. All attempts to demonstrate a greater effect of one pole in these expansion effects resulted negatively. . . . In two cases out of ten experiments with bits of isolated skin it was thought that the expansion at the cathode began earlier and was more extensive than at the anode, but this is not sufficient evidence for ascribing to that pole a greater effect.

From previous experiments with a variety of physiological stimuli (Spaeth, 26) I have been led to believe that the contracted phase of the melanophore is that of stimulation. It seemed strange, therefore, that a constant current should produce a *relaxation* in amphibian melanophores. Believing the polarizable electrodes of Laurens and Winkler[6] to be a possible source of error in their experiments, I first made a number of preliminary trials with *Fundulus* melanophores using a constant current delivered through platinum electrodes. Scales immersed in 0.1 N KCl were subjected to galvanic stimulation from one, two and three 2-volt storage cells. The first experiment showed that, under these experimental conditions, a relaxation of the melanophores begins immediately. In subsequent experiments a drop of neutral red added to the KCl solution showed, furthermore, that the expansion invariably begins at the cathode. Under the low power of the microscope it was a simple matter to observe the color change of the neutral red at the cathode. The yellow area of alkalescence gradually diffused toward the anode and a short distance behind the periphery of the yellow semicircle the melanophores could be seen to be expanding. By the time the alkaline region had extended to the anode all the melanophores were either fully expanded or showed a distinct beginning of an expansion. When a scale was transferred from the now alkaline KCl solution to neutral KCl all the melanophores on the side of the scale towards the anode contracted completely. Thus in the region where the exposure to OH ions had been shortest and where the hydroxyl concentration was at a minimum the relaxation was reversible. All the melanophores in the region of the cathode, however, remained expanded and soon disintegrated (fig. 5). Fifteen minutes after having been returned to neutral KCl the scale appeared half dark and half light.

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[6] Presumably Winkler used polarizable electrodes since he makes no statement to the contrary.

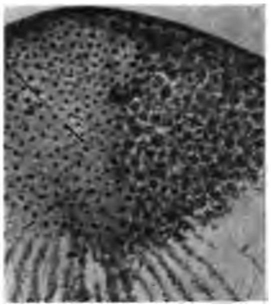

FIG. 5. Median portion of a scale showing the effect of stimulating with a constant current through platinum electrodes placed on either side of the scale. The melanophores towards the cathode are expanded by the action of the liberated OH ions.

From this first set of preliminary experiments it seemed that when the melanophores are stimulated with a galvanic current, using platinum electrodes, the relaxation that appears is due, *not* to the electrical stimulus, but to the effect of OH ions liberated at the cathode. The following experiments supported this conclusion. Two depression slides were placed side by side, filled with ½ cc. 0.1 N KCl and connected by a narrow bridge of filter paper, supported by a cover glass set on edge between the slides. A scale with contracted melanophores was now placed in each chamber. After adding a small drop of aqueous neutral red solution to the KCl solution in each chamber, a platinum electrode was brought into contact with the bony portion of each scale. A constant current (three storage cells) was now passed for five minutes from one scale over the moist filter paper bridge to the other. The diffusion from the cathode to the anode was largely prevented by the filter paper bridge. In about two minutes all the melanophores of the scale in the cathode chamber were fully relaxed, while those in the anode chamber remained contracted. The neutral red solution had meanwhile turned yellow at the cathode and was becoming colorless at the anode on account of the bleaching effect of the liberated Cl_2 or O_2. At the close of the experiment, the scales and the electrodes were removed and, without disturbing the solutions in the two depression slides, a fresh scale with contracted melanophores was brought into each chamber. Fifteen minutes later, no current having passed meanwhile between the chambers, all the melanophores of the scale in what had previously been the cathode chamber were completely expanded while those of the scale in the anode chamber remained contracted. The two scales were thereupon fixed in absolute alcohol. They are shown in figures 6 and 7.

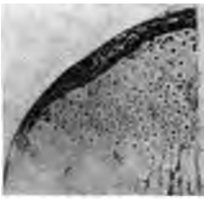

Fig. 6

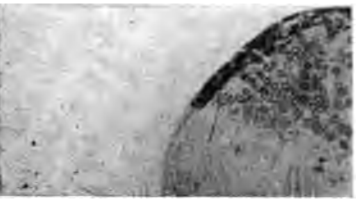

Fig. 7

Fig. 6. Anodal products of electrolysis of KCl solution give no relaxation. See text for details.

Fig. 7. Hydroxyl ions produced at the cathode cause relaxation of melanophores in KCl. See text for details.

From these two experiments it is evident that the expansion of melanophores which is initiated at the cathode upon stimulation with a galvanic current and polarizable, platinum electrodes is effected by the OH ions and not by the passage of the constant current through the cells. Finally, experiments were carried out with solutions of KOH in 0.1 N KCl which showed that, beyond a definite OH concentration, a relaxation of the melanophores invariably occurs.

THE EFFECT OF THE CONSTANT CURRENT ON SMOOTH MUSCLE

Practically all experimenters[6] on smooth muscle preparations agree that both the make and the break of the constant current act as stimuli and produce contractions. The make contraction is larger than the break. Stewart (l.c.) has shown conclusively that in the cat's bladder the *flow* of the current also acts as a contracting stimulus. In his experiments the current (about 3 m.a.) was made, allowed to flow for a few seconds, and then broken. The result was a strong make contraction succeeded by a period of very slow, imperfect relaxation, the true relaxation occurring only after the break contraction. That the tonic elevation was actually due to the flow of the current was shown by making the current in the same way but shutting it off by means of a rheonome. In this way the influence both of flow and of break was eliminated. The curve showed a strong make contraction followed by a complete relaxation.

In spite of Stewart's proof to the contrary the impression continues to exist that smooth and striated muscle are alike in their response to the constant current. Thus du Bois Reymond (5) states (p. 554):

> Bei der Reizung mit dem konstanten Strom gilt zunaechst wie bein gestreiften Muskel, dass nur Schliessung und Oeffnung, jede als ein einzelner Reiz, wirksam sind, waehrend die Durchstroemung an sich keinen Reiz bildet.

In the case of the melanophores of *Fundulus* no response has as yet been observed to the breaking of the constant current, but both make and flow cause a contraction, as was the case in Stewart's experiments on the cat's bladder.

I have recently carried out a series of experiments upon the melanophores of *Fundulus* using non-polarizable electrodes of the Zn-$ZnSO_4$ type. Stimulation was effected by means of fine camel's hair brushes set in plaster caps covering the ends of the heavy glass tubes containing the concentrated solution of zinc sulphate. The plaster caps were made up fresh for every experiment or short series and in no case were the electrodes used longer than two hours at a time. The plaster was mixed with 0.1 N NaCl solution and, after setting, both electrode caps were saturated with 0.1 N NaCl. As an additional precaution the NaCl from the diffusion chamber, in which an experiment had been carried out, was tested for SO_4. The non-polarizable electrodes were most conveniently manipulated by having their adjustable uprights mounted on a sheet of glass (figure 3 of the previous paper in this number of this JOURNAL.) A depression slide or shallow diffusion slide containing about 0.5 cc. 0.1 N NaCl and a scale with expanded melanophores was brought below the objective on the sheet of glass on the stage of the microscope. The electrodes were then swung into position and the final adjustment of the single melanophore to be studied was accomplished by moving the sheet of glass carrying both the electrodes and the diffusion chamber. The direct current of the city circuit was reduced by means of a long tube containing one movable and one fixed copper electrode and a dilute solution of copper sulphate. The strength of the current was varied by sliding the movable electrode back and forth in the tube. The actual current that stimulated the single cell could not be accurately measured since the electrodes simply dipped into the solution containing the melanophores.

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[6] Gruetzner (l. c.) gives an extended review of the older papers on this subject.

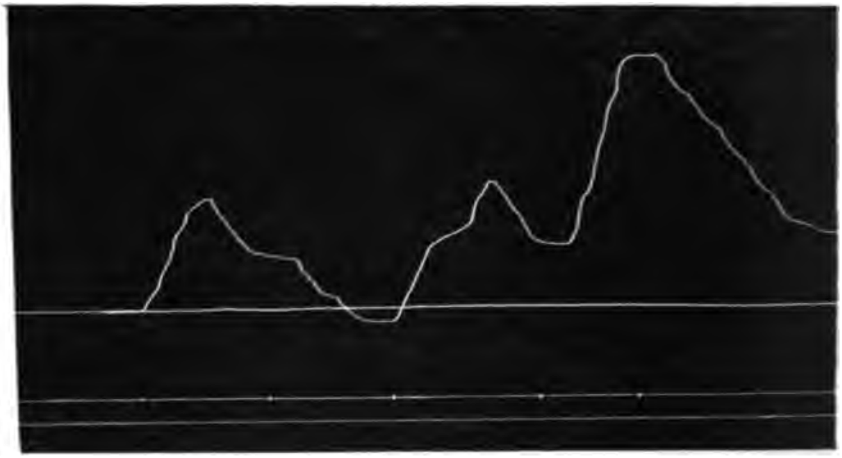

FIG. 8. Effect of a weak constant current through non-polarizable electrodes. Stimulation began 45 minutes after removing the cell from the fish to 0.1 N NaCl. Time in seconds. $T = 21.3°C$. Detailed explanation in text.

The responses of the melanophores to a constant current of medium strength show a striking correspondence to the reactions of smooth muscle preparations under similar experimental conditions (fig. 8).

There is a sharp rise at the make after a very brief latent period

(<2 seconds) followed by a gradual and partial falling off of the contraction, giving a characteristic plateau. Stewart (fig. 9, p. 196, l.c.) recorded precisely this type of curve for the response of the cat's bladder. He noted further a distinct response in the bladder preparation when the constant current was broken, which I have never observed in the melanophore. There is, however, a latent period of about 10 seconds before the relaxation begins which may represent a suppressed influence of the break shock.

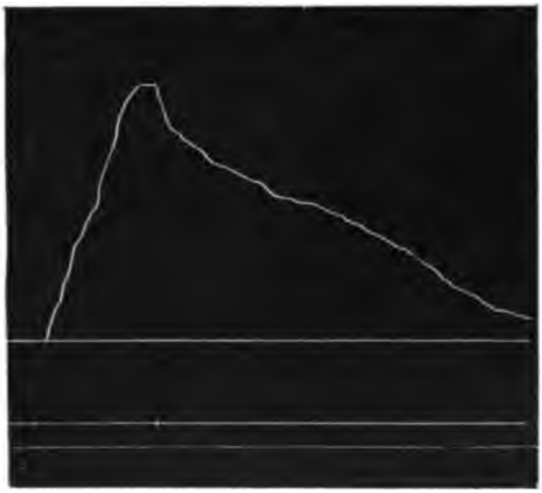

FIG. 9. Curve showing the response of a melanophore to a strong constant current through non-polarizable electrodes. A complete contraction occurs which continues during the *flow* of the current.

The second part of figure 8 represents the effect of the flow of the constant current. A stimulus of medium strength was sent into the preparation and, after the plateau had appeared (at the fourth signal), the current was gradually increased by means of the sliding electrode of the copper sulphate rheonome. In this case there was no additional make stimulus, yet the melanophore contracted completely and remained contracted during the flow of the stronger current. At the fifth signal the current was broken and a gradual expansion of the pigment followed.

When the stimulus is sufficiently strong the melanophore contracts completely and remains contracted until the breaking of the current.

This condition is illustrated in figure 9, which shows the effect of a strong constant current. The latent period is again very short (<2 seconds) and the contraction persists during the flow of the current, as is shown by the flat top of the curve. In this case the contraction represents the response to a combination of make and flow stimuli.

DISCUSSION OF RESULTS

The striking correspondence between the curves recorded here and the contraction curves of a variety of smooth muscles would not in itself be significant. The pseudopodia of an amoeba or a heliozoan might exhibit contraction phenomena of the same general order. However when this correspondence is considered in conjunction with other morphological and physiological characters of the melanophore such, for example, as the sympathetic innervation, the sensitivity to adrenalin and certain other drugs, the power of rhythmic pulsation, the ability to maintain a condition of increased tonus for protracted periods,[7] etc., we are justified in attaching greater significance to the similarity.

In view of a considerable amount of verbal criticism regarding the earlier contention that the melanophore is to be considered a functionally modified smooth muscle cell it may be well to include here a brief statement of the author's position.

We apply the term "smooth muscle" to a variety of tissues which correspond to a number of empirically selected morphological and physiological characteristics. Investigation has shown that the melanophore conforms to *all* the criteria by which we recognize smooth muscle. Since the empirical criteria that establish the category of smooth muscle and the universal characteristics of smooth muscle are identical, it follows that the melanophore exhibits all the characteristics of smooth muscle. Hence it seems logical to speak of the melanophore as a type of smooth muscle cell. The insistence upon the nomenclature is significant in so far as further study of the melanophore may throw light upon certain obscure phases of the problem of muscle contraction.

SUMMARY OF RESULTS

1. The melanophores of *Fundulus heteroclitus* fail to contract completely when stimulated by a single make or break induction shock.

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[7] The melanophores in a living fish will apparently remain light over a light bottom indefinitely. I have kept *Fundulus* in a white aquarium for several months and have observed no tendency to turn dark during this period.

2. A tetanizing current of moderate intensity produces a complete contraction in which the rising phase lasts 25 to 30 seconds and the relaxation 65 to 90 seconds. Contraction and relaxation are completed in 90 to 120 seconds.

3. The form of the contraction curve is never smooth as in a curve of typical muscular contraction for the pigment granules exhibit a certain amount of irregularity and blocking in their migration within the cell processes.

4. The contraction wave originates at the center of the cell and passes to the periphery at the rate of about 22 μ per second.

5. The most rapid pigment migration thus far observed in response to electrical stimulation is 3 μ per second.

6. With a moderate tetanizing current there appears a latent period of about 5 seconds at the distal ends of the cell processes whereas nearer the proximal ends of the processes the latent period is only about 2 seconds.

7. With an increase in the strength of the stimulus there appears a progressive shortening of the latent period and an increase up to a maximum in the height of the contraction.

8. By the proper spacing of single break shocks which are individually almost or entirely ineffective as contracting stimuli, there appears a summation of stimuli. Eventually, with an interval of 2 to 3 seconds, complete fusion occurs, giving a typical tetanus curve.

9. The relaxation of the melanophores observed by previous investigators upon stimulation with a constant current and polarizable electrodes is probably brought about by the hydroxyl ions liberated at the cathode and is not the result of galvanic stimulation.

10. By using non-polarizable electrodes it appears that both the make and the flow of the constant current act as contracting stimuli. No response to the breaking of the constant current has thus far been observed.

11. In its responses to electrical stimulation the melanophore shows all the characteristics of smooth muscle. The experiments recorded in this paper thus corroborate and amplify the author's former conclusion that the melanophore is to be considered a functionally modified type of smooth muscle cell.

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A DEVICE FOR RECORDING THE PHYSIOLOGICAL RESPONSES OF SINGLE MELANOPHORES

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In a previous paper[1] I have figured a curve showing the rhythmic pulsations of a single melanophore of *Fundulus* following a ten minute immersion in a 0.1 N solution of $BaCl_2$. This curve was obtained by measuring, with an ocular micrometer scale, the greatest diameter of a melanophore in the course of an hour, at intervals of from ten to thirty seconds. These empirical diameters were plotted against the time, giving a curve of pulsation. By using several microscopes with similar micrometer eye-pieces it was a simple matter to compare the effects of different stimulating solutions upon melanophores of the same fish. When the experiments lasted several hours and the movements of the pigment granules were relatively sluggish, this method proved more convenient than the automatic method described below. Frequently, however—as for example, in the case of a contraction by KCl or an induction current—the responses were so rapid that it became impossible to obtain an exact record of the pigment migration in terms of a series of diameters.

In order to obtain more accurate records of the physiological activities of the melanophores, the following graphic method was devised. The movements of the pigment granules in a single process of a melanophore were followed with an ocular micrometer carrying an adjustable scale. A cell was selected in which the processes were relatively long and straight. A single process was then brought to lie at right angles to the division of the micrometer scale with an empirically selected scale division just tangent to the outermost pigment granules (fig. 1a). When distal and proximal movements of the pigment granules occurred they were easily followed by turning the adjustment screw of the ocular micrometer. By means of a thin belt the turns of the adjusting screw

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[1] Spaeth: Journ. Exper. Zöol., 1916, xx, 193.

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by means of a mechanical stage which carried the entire chamber. All observations were made with a 7 Leitz objective used as a water immersion lens.

In order to maintain a constant base line it was necessary to devise a mechanism for holding the melanophores immovably in position throughout the period of observation. This was accomplished as follows. A piece of fiber-composition was cut to span one end of the diffusion chamber, leaving a short flange projecting over each side. By means of a horizontal set screw (fig. 2 b) in one of these flanges, the bridge was firmly clamped across one end of the chamber. In the middle of the bridge a vertical set screw (fig. 2 c) raised or lowered the free end of a thin strip of spring brass (fig. 2 d) attached to the lower surface of the bridge. The free end of the brass strip carried a small two-pronged fork made of heavy platinum wires. Figure 2B shows how a scale is held in place against the bottom of the diffusion chamber by means of the flattened tips of the platinum wires. The platinum wires serve not only as a clamp for holding the scale with the melanophores in position but also as electrodes for faradic stimulation. Short pieces of copper wire were fused in a blast lamp to the proximal ends of the platinum wires and attached to two binding posts (*F*) on the fiber bridge. The two platinum wires were mutually insulated by means of a short glass rod lying between them. This rod was attached to them by cement and was similarly fastened to the free end of the spring brass. In order to avoid, as far as possible, the short circuiting of the secondary current through the salt solution between

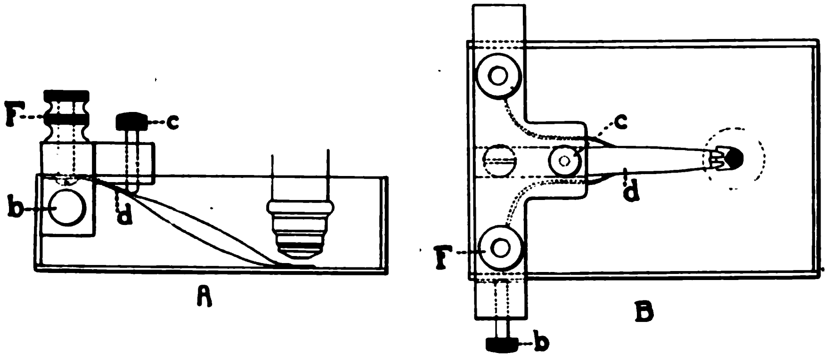

FIG. 2. Diffusion chamber and method of holding a scale in place during an observation. See text for details.

the prongs of the fork, the flattened ends of the platinum wires, with the exception of the lower surface of the tips, were coated with a thin layer of cement.

A second type of diffusion chamber was found to be very convenient in experiments demanding a rapid change of solutions. This consisted of a circular glass chamber with glass inlet and outlet tubes mounted on a heavy slide. The scale was kept in position in the middle of the circular chamber by means of a small piece of cover glass about 3 by 8 mm., one end of which rested upon the free, bony portion of the scale. By a touch of vaseline[2] the other end was made to adhere firmly to the bottom of the chamber. This arrangement was found particularly useful when stimulating with non-polarizable electrodes.

The movement of the ocular micrometer scale is normally accomplished by turning a milled head. This head was replaced by a small brass crank which enabled the operator to obtain a continuous and smooth motion of the micrometer scale. The rotation of the ocular micrometer screw shaft was transmitted to a set of wooden pulleys by a light belt. A piece of three-strand fish line with the ends spliced together gave very satisfactory results. The pulleys were so arranged that, in addition to the three transmission speeds, there were two winding spools for the cord carrying the heart lever. Various combinations of transmission speeds and winding pulleys gave magnifications on the kymograph drum ranging from 940 to 3850 diameters.

The device for carrying the writing lever was constructed as follows. A lead block (fig. 3, *L*) about 4.5 cm. long, carrying a heart lever, was arranged to run on parallel brass rods (*R*) the ends of which were screwed into a small iron base. A heavy piece of sheet brass (*S*) was soldered across the upper ends of the brass rods. By maintaining a constant distance between the rods, any possibility of sticking or of lost motion in the lead block was prevented. Through a hole in the middle of the brass piece (*S*) a cord (*C*) was passed and attached to an eye in the lead block. The other end of this cord was fastened to the winding pulley by a touch of sealing wax. By turning the adjusting screw of the ocular micrometer, the lead block carrying the writing lever was raised and lowered by the winding and unwinding of the cord attached to the pulley axis. The advantage of this arrangement as compared to an ordinary heart lever lies in the fact that since the writing tip moves in a plane perpendicular to the base line, every point of the contraction curve may be projected to the time-interval line, and, since it is unnecessary to apply corrections for a lever arc, the progress of the experiment may be read directly.

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[2] The vaseline has no injurious effect on the melanophores.

In experiments with salt solutions it was found that better results were obtained by momentarily raising the objective from the surface of the scale when the solution was being changed. The raising of the objective would obviously release the tension on the transmission belt, and the weight of the lead block would move the writing lever out of position. In order to maintain the position of the writing point, even when the objective was raised above the surface of the solution, a spring brake was attached to the pulleys, adjusted to overcome the rotary tension of the lead weight at the pulley axis.

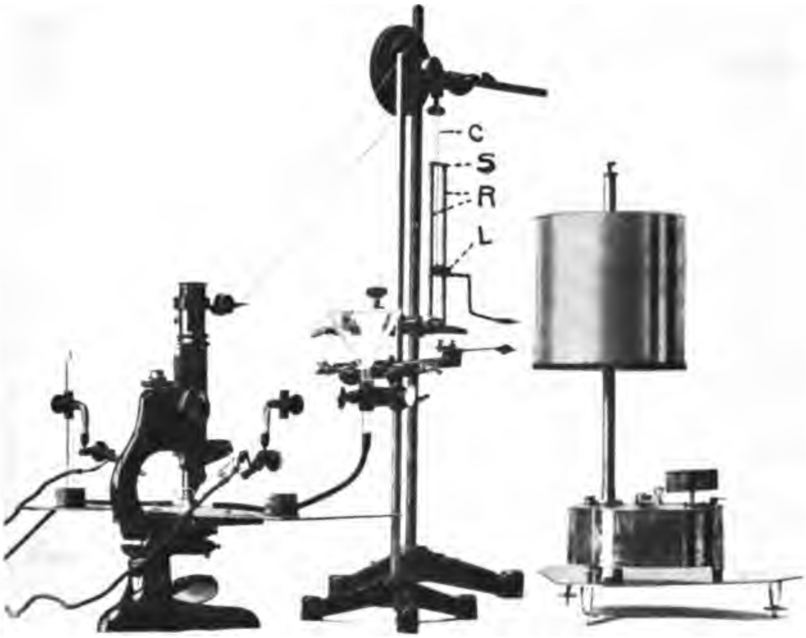

FIG. 3. Recording apparatus arranged for stimulation with non-polarizable electrodes. Letters explained in text.

At the beginning of an experiment the time and stimulation signals were aligned with the moveable writing lever as follows. The tip of the writing lever having been brought into contact with the lower edge of the drum, the eye-piece micrometer screw was turned until the writing lever had traced a single vertical line upon the smoked paper. The time and stimulus signals were now brought into contact with the kymograph paper with their tips on this vertical line. The angle of the vertical line with the base line was adjusted to 90° by means of a levelling table carrying the kymograph.

The most obvious disadvantage of this apparatus is that it requires constant attendance and manipulation by the operator, which, in addition to being time-consuming and laborious, introduces a personal equation into every observation. In most cases, however, the reactions are relatively so slow that, with a little experience, the pigment may readily be followed with the micrometer scale division. The advantage of the apparatus lies in that it affords an opportunity for recording graphically the physiological responses of single melanophores. Such graphic records and a discussion of their significance in the problem of muscle contraction will be found in another paper in this number of this journal.

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